British Medical Bulletin

Reactions to Injury

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BOOK REVIEWS

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Scientific Editor: *W. G. Spector*

Reactions to Injury

Wars in the present century greatly stimulated investigation into the effects of injury on the body and treatment of such injuries. Not only was the harvest of knowledge gained by research in the Second World War exceptionally great, but, as will be seen from the present symposium, the impetus then given is still maintained. The result has been a vast improvement not only in the treatment of war wounds, but also of accidents of civilian life. This has necessitated the maintenance of a large and active blood transfusion service during peace as well as war. How great has been the improvement in wound therapy can be seen in the amazing figures of recovery of wounded men in the advance through Normandy and Germany of the allied forces in 1944–45 and more lately in Korea.

It is unfortunately true that the public are much more interested in lethal processes, such as the atom bomb, than in life-saving discoveries, and there is but little popular appreciation of the revolution that has resulted from increased knowledge of treating injured people in the last ten years. The present symposium, *Reactions to injury*, gives an opportunity to the reader to understand the scientific basis of these important practical advances in wound therapy. He may not get the impression from this symposium of the degree of successful achievement, for it is the way of the scientific worker not to emphasize this so much as the problems and the difficulties which still beset him. In the case of trauma, these difficulties remain great, especially that of getting insight into the precise mechanism whereby injuries produce such widespread depression in functional activity of many remote organs and tissues. It is, however, obvious that, as in past triumphs, future progress in combating the effects of injury must depend on the acquisition of further knowledge. For those interested in the advances of this branch of medical science, and especially for those about to enter this field of study, there could be no better starting-off ground than the present volume, in which the articles are written by experts and by those who have contributed substantially to the discoveries described.

The most prominent and feared reaction that often follows injury is traumatic shock. The manifestations of this condition vary, and the attempts of medical men to define shock have been so indecisive that some people use the word with reluctance. Still there is no doubt about its reality and its dangers. The known outstanding features of traumatic shock are the changes in the circulation and chemistry of the blood and in the distribution of body fluids generally. Practically all modern treatment of the condition is directed to the correction of these factors. That this is so can be seen in the great attention given to the problem of blood transfusion in the present volume. This aspect of the subject has been described by Dr R. T. Grant who has had unrivalled experience in both studying and treating traumatic shock under conditions of war. Another paper on circulatory defects produced by injury and their treatment is that of Dr R. Bodley Scott. Whereas these articles deal primarily with the clinical problems of abnormalities of the circulation resulting from trauma and their treatment, a scientific discussion of the changes in the fluid distribution of the body, and especially the shifting of the fluid from the intravascular to the extravascular spaces resulting from injury, is given by Dr F. C. Courtice.

The continuation of the impetus given by war conditions to research on blood transfusion is also seen in the series of articles on the provision of materials for transfusion after injury, the foreword to which is contributed by Sir Alan Drury. In this case, however, attention has been directed to the cytology and chemistry of the blood and to plasma substitutes to be used in transfusions. It is satisfactory to learn that Dr P. L. Mollison has found that red blood corpuscles can now be conserved in a normal condition for at least six months if stored at −79° C., and it is hoped that this new method will lead to important improvements in the storage of blood constituents for purposes of transfusion. Dr M. E. Mackay has provided an account of two of the plasma products fractionated by the new ether method. These deal respectively with the clinical use of serum albumin and with the preparation and use of fibrin foam, rich in thrombin, as a haemostatic in neurosurgical operations and skin grafting. Dr W. d'A. Maycock has written a most useful account of the relative value and limitations of plasma substitutes such as dextran, gelatin and polyvinylpyrrolidone for transfusion purposes.

It will be generally agreed that future progress in the treatment of traumatic shock depends on the acquisition of more knowledge of the biochemical changes which are interposed between the time of the injury and the development of shock and other morbid manifestations. The articles in the present volume, of Dr D. P. Cuthbertson on the "Interrelationship of Metabolic Changes Consequent to Injury," of Professor H. N. Green & Dr H. A. Stoner on the "Effects of Injury on Carbohydrate Metabolism and Energy Transformation" and of Drs J. D. Judah & W. G. Spector on the "Reaction of Enzymes to Injury", are evidence that this fundamental aspect of the subject is receiving the attention that it demands. The first two of these articles emphasize the profound metabolic changes which result from trauma, and lea.e but little doubt that work of this nature will ultimately lead to the discovery of unknown and vital mechanisms of metabolic control. Until this knowledge is acquired, treatment by transfusion will certainly maintain its dominant position in shock therapy.

Besides shock, injury brings with it other complications which may be much more serious than the injury itself. Of these, invasion of wounds by micro-organisms has always been the most dangerous to life until recent times. It is indeed an indication of one of the triumphs of modern medicine, namely, the success of combating wound infection, that so little space is given to this subject in the present volume. In spite of the great advances in chemotherapy, both tetanus, which is described by Professor G. Payling Wright, and gas gangrene, described by Professor C. L. Oakley, remain most serious complications of injury if they once develop. It was shown in the Second World War that, on the basis of knowledge already obtained, tetanus could be practically eliminated by prophylactic treatment with tetanus toxoid. Professor Wright has indeed advocated the mass extension of tetanus prophylaxis to the civil population. Professor Oakley has made it clear that modern medical treatment, either prophylactic or curative, is not yet sufficiently successful in the case of gas gangrene and that early and adequate surgery still remains a sine qua non for dealing with this condition.

Trauma is only one cause of bodily injury, and attention has been given in the present volume to other causes, such as burns and war gases, which are discussed by Dr J. P. Bull and Dr H. Cullumbine respectively. Dr Bull has had the advantage of working with Dr L. Colebrook at

the Medical Research Council's Burns Research Unit at Birmingham, where the systematic study of the treatment of burns has led to important advances in therapy. War gases, whether used as lachrymators, skin irritants or vesicants, lung irritants or lethal gases, remain at least potentially a major problem of war, and the light thrown by recent research on the interaction of some of these with known enzyme systems is of real scientific significance.

Two of the articles in the present volume deal with injuries to the nervous system, namely, "Studies on Head Injury" by Dr W. Ritchie Russell, and "Demyelination" by Dr J. B. Cavanagh & Professor R. H. S. Thompson. In the first of these, Dr Russell has contributed a clinical account of concussion which will be appreciated by all who have to deal with this complication of brain injury. The article on demyelination, on the other hand, is a scientific study of nerve degeneration and is a reminder that, apart from trauma, this may be the result of many unsuspected causes, and that in the case of neurones of the central nervous system, some of these, which have long been a mystery, are beginning to be understood.

It has only been possible in this preface to give a rough outline of the reactions of the body to injury as presented by the present band of authors. Perusal of the articles themselves with their mass of valuable information and scholarly discussion will soon reveal how well they have described the present position of knowledge, both practical and academic, of this subject.

Edward Mellanby

We are grateful to the members of the planning committee, Sir Howard Florey, Professor G. R. Cameron and Dr R. T. Grant, for their help in arranging this symposium.

Notes on Contributors

DR J. P. BULL is Director of the Medical Research Council's Industrial Injuries and Burns Research Unit at the Birmingham Accident Hospital. After a period of research work on casualties caused by bombing, he served in the Royal Army Medical Corps from 1942 to 1946. He has recently been working on various problems of the pathology and treatment of burns and other injuries. His published work includes studies on the impairment of sensation in burns, improvements in dressings, dextran as a plasma substitute, industrial injuries, and the treatment of burns and their mortality. An article by Dr Bull, entitled "Industrial injuries", appeared in *British Medical Bulletin*, 1950, **7**, 69.

DR J. B. CAVANAGH was graded Pathologist Captain in the Royal Army Medical Corps from 1946 to 1948. He was formerly Demonstrator in Pathology, and subsequently Senior Registrar in Pathology at the Bernhard Baron Institute of Pathology, London Hospital, where he took a special interest in neuropathology. He was recently appointed Research Fellow in Neuropathology at Guy's Hospital, London, to work on problems associated with demyelination and allied conditions.

DR F. C. COURTICE is Director of the Kanematsu Memorial Institute of Pathology at Sydney Hospital, and was formerly Reader in Human Physiology at Oxford University. During the Second World War he was attached to the Chemical Defence Experimental Station at Porton, Wiltshire. His earlier work dealt with general metabolism of exercise (*Proc. roy. Soc.* B, 1936, **119**, 381), the cerebral circulation (*J. Neurol. Psychiat.* 1940, **3**, 293), respiration (*J. Physiol.* 1947, **105**, 345) and the functional disturbances caused by various toxic agents. His present work is mainly concerned with the physiology of the circulation and of the body fluids, especially with the functional significance of the lymphatic system.

DR H. CULLUMBINE is a graduate in physiology and in medicine of the University of Sheffield. During the Second World War he was seconded, as a lieutenant-colonel, to the Ministry of Supply for research work. Between 1947 and 1951 he was Professor of Physiology and Pharmacology, University of Ceylon, Colombo. He is a member of the Advisory Panel on Nutrition to the World Health Organization and Honorary Consultant in Experimental Physiology to the Army. At present Dr Cullumbine is a member of the scientific staff of the Ministry of Supply. He is the author of over 60 publications on various physiological, nutritional and pharmacological subjects.

DR D. P. CUTHBERTSON was appointed Director of the Rowett Research Institute, Bucksburn, Aberdeenshire, in 1945 in succession to Sir John—now Lord—Boyd-Orr. He was also appointed at that time Consultant Director of the Commonwealth Bureau of Animal Nutrition. Dr Cuthbertson is also Honorary Consultant in Nutrition and Physiology to the Army. He was Lecturer in Pathological Biochemistry at the University of Glasgow, and Clinical Biochemist to Glasgow Royal Infirmary from 1926 to 1934, when he was appointed Grieve Lecturer in Physiological Chemistry in the University of Glasgow. From 1943 to 1945 he was seconded to the headquarters staff of the Medical Research Council from the University of Glasgow. During his period of service with the Medical Research Council he was secretary to their committees on traumatic shock, blood transfusion research, haemoglobin surveys, and protein requirements. In 1950 he was a member of the United Kingdom Agricultural Mission to Canada. During the past quarter of a century Dr Cuthbertson's personal research work has been mainly directed to the study of the metabolic responses to injury, factors influencing wound-healing and the strength of bone, and to the interrelationships of protein and carbohydrate metabolism. He has contributed two previous articles to the Bulletin: the first, on protein metabolism, appeared in *British Medical Bulletin*, 1944, **2**, 207; the second, on the physiology of convalescence after injury, was published in *British Medical Bulletin*, 1945, **3**, 96. His article in this number of the Bulletin briefly surveys the interrelationship of the metabolic changes consequent to injury.

SIR ALAN DRURY has been, since 1952, on the Scientific Staff of the Agricultural Research Council. From 1943 to 1952 he was Director of the Lister Institute of Preventive Medicine, London, and prior to this a member of the Scientific Staff of the Medical Research Council. He commenced his scientific career in association with Sir Thomas Lewis and studied problems connected with cardiology. In 1926 he moved to Cambridge and worked on substances of biological interest present in tissues and blood. He was made Huddersfield Lecturer in Special Pathology, and was elected to the Fellowship of the Royal Society in 1937. In 1939, Sir Alan Drury joined the headquarters staff of the Medical Research Council and was intimately connected with special war units dealing with blood transfusion. He is Chairman of the Blood Transfusion Research Committee. He has published a number of papers dealing with cardiological problems and with adenosine and psychosin.

DR R. T. GRANT has been working under the Medical Research Council since 1921—with Sir Thomas Lewis at University College Hospital, London, until 1934, and since then as Director of the Medical Research Council's Clinical Research Unit at Guy's Hospital, London. His work has been the study of the cardiovascular system in health and disease. During the Second World War Dr Grant made clinical studies on patients suffering from "wound shock" during the air raids in England and on the battlefield in Italy. The results of these studies, written in collaboration with E. B. Reeve, were published in *Observations on the general effects of injury in man with special reference to wound shock* (*Spec. Rep. Ser. med. Res. Coun., Lond.* No. 277, 1951). He was elected a Fellow of the Royal Society in 1934.

PROFESSOR H. N. GREEN recently held the Chair of Pathology in the University of Sheffield. He is now Professor of Experimental Pathology at the University of Leeds and Director of Cancer Research at the Universities of Leeds and Sheffield. He has worked in a wide field of experimental medicine, aiming throughout at a close liaison between the laboratory and the ward. His early researches in the laboratories of Sir Edward Mellanby at Sheffield were mainly concerned with nutrition, and he published a variety of papers dealing with diet and infection, diet and pregnancy, and the biochemistry of vitamins A and D. Later he became a demonstrator at the University of Cambridge and there, whilst working on bactericidal factors in the blood, became interested in chemotherapy. Professor Green has contributed in several papers to our understanding of the mode of action of sulphonamides and related compounds. During the Second World War he was asked by the Medical Research Council to

NOTES ON CONTRIBUTORS

organize a clinical team to study traumatic shock. These investigations led to pure laboratory studies in which the shock-inducing action of adenosine triphosphate was discovered. These, and related studies, culminated in a book, written jointly with Dr H. B. Stoner, *Biological actions of the adenine nucleotides* (1950). Professor Green has previously contributed to *British Medical Bulletin*, 1945, **3**, 102, an article on biochemical factors in the aetiology of "shock". He was Director of the University of Sheffield Cancer Research Laboratories for several years, and reports of his many investigations are to be found in papers and in the Annual Reports of the British Empire Cancer Campaign from 1937 to 1953.

Dr J. D. Judah is Lecturer in the Department of Morbid Anatomy at University College Hospital Medical School, London. He has devoted most of his scientific career to the study of cellular metabolism and to the application of enzymological techniques to problems of pathology and toxicology. He has published papers, singly and in collaboration, on cell metabolism and on the effects on cell metabolism of insecticides, dyestuffs, 2:4-dinitrophenol and carbon tetrachloride. He is at present engaged, together with Dr K. R. Rees of the Department of Biochemistry, University College, London, on studying the metabolism and metabolic requirements of the central nervous system.

Miss Margaret E. Mackay was as a research student in the Department of Biology, University of Adelaide, graduating in 1933, and M.Sc. six years later. She worked with the Medical Research Council's Drying Unit in the Department of Pathology, University of Cambridge, from 1939 to 1942, when she obtained her Ph.D. degree. Her work at this time was chiefly concerned with the bacteriological control of human serum and plasma for freeze-drying. From 1942 Miss Mackay has been on the Medical Research Council External Scientific Staff, until 1952 with the Blood Products Unit at the Lister Institute of Preventive Medicine, London, and now attached to the Division of Biophysics at the same Institute. Since 1942 her work has been in collaboration with Dr R. A. Kekwick, and others, on the isolation and characterization of biologically active fractions from human and animal serum and plasma. She is joint author with Dr R. A. Kekwick of *The separation of protein fractions from human plasma with ether* (In: *Spec. Rep. Ser. med. Res. Coun., Lond.* No. 286, in press).

Dr W. d'A. Maycock, after a period spent in physiological research, served during the Second World War in the Royal Army Medical Corps. At the end of the war, he joined the Scientific Staff of the Lister Institute of Preventive Medicine, Elstree, Hertfordshire, where he is now in charge of the Blood Products Laboratory. He is Adviser on Blood Transfusion to the Ministry of Health, and Honorary Consultant in Blood Transfusion and Resuscitation to the War Office. His published work is mainly concerned with shock and with plasma substitutes.

Sir Edward Mellanby, G.B.E., K.C.B., F.R.C.P., F.R.S., who writes the Introduction to this symposium, was a Member of the Medical Research Council from 1931 to 1933, when he became Secretary of the Medical Research Council, a position he held for 16 years. Formerly he was Professor of Physiology, University of London; and Professor of Pharmacology, University of Sheffield. He was Chairman of the Nutrition and Vitamin Standardisation Committees of the League of Nations in 1931 and 1934. He has been awarded medals and prizes by many universities and other learned bodies, including the Royal Society, and holds honorary degrees from British and foreign universities. Sir Edward Mellanby's publications include *Nutrition and disease* (1934), *A story of nutritional research* (1950), and the Medical Research Council's Special Reports, Nos. 61 and 93, on experimental rickets (1921 and 1925), and also many scientific papers.

Dr P. L. Mollison has, since 1946, been at the Postgraduate Medical School of London as Director of the Medical Research Council's Blood Transfusion Research Unit and as Lecturer in the Department of Medicine. He is at present engaged in the search for satisfactory methods of storing red blood cells at temperatures below 0° C. He began work on blood transfusion in 1940, when, with Miss I. M. Young and later with Dr J. F. Loutit, he studied methods of red-cell preservation. This work led, firstly, to the recognition that tests made in vitro on stored red cells were unreliable guides to survival in vivo, and secondly, to the recognition that acid-citrate-dextrose solutions were better than any previously described solutions as red-cell preservatives. The technique of differential agglutination was next applied to a study of the haemolytic anaemias. With Dr J. V. Dacie, in 1943, he described cross-transfusion experiments in hereditary spherocytosis and showed that, in patients with this disorder, transfused normal red cells survived normally, whereas the patient's own red cells had a diminished life-span. Between 1947 and 1951 Dr Mollison studied the natural history of haemolytic disease of the new-born and the effects of treatment, and organized controlled trials which showed decisively the value of exchange transfusion. In 1950, with Miss M. Cutbush and Dr D. Parkin, he described a new blood-group system (the "Duffy"); he has published various papers relating to blood-group incompatibility. He is author of *Blood transfusion in clinical medicine* (1951) and is a joint author of papers on the in-vivo survival in the human subject of transfused erythrocytes after storage in various preservative solutions (*Quart. J. exp. Physiol.* 1942, **31**, 359) and on the survival of normal erythrocytes after transfusion to patients affected with familial haemolytic anaemia (*Lancet*, 1943, **1**, 550).

Dr C. L. Oakley is Brotherton Professor of Bacteriology in the University of Leeds, to which he recently moved from the Wellcome Research Laboratories, Beckenham, Kent, where he was Head of the Department of Immunology and Experimental Pathology. He has written extensively on the soluble antigens of the clostridia and on the sites of production of antibodies.

Dr W. Ritchie Russell is Director of the Department of Neurology, United Oxford Hospitals, Consultant Neurologist to the Army and formerly to the Ministry of Pensions, and Hon. Editor of the *Journal of Neurology, Neurosurgery and Psychiatry*. He graduated M.B., Ch.B. in Edinburgh University in 1926 and, after house appointments at Edinburgh Royal Infirmary, he held in succession the posts of Resident Medical Officer, National Hospital, Queen Square, London; Clinical Tutor, later Assistant Physician, Edinburgh Royal Infirmary; Lecturer in Neurology, Edinburgh University. During the Second World War he was Specialist in Neurology and Consultant Neurologist to the Middle East Forces. Since 1945 he has worked in Oxford. Since 1932 Dr Ritchie Russell has written many papers on subjects related to the effects of head injury. Other published studies include those on the treatment of painful amputation stumps and phantom limbs; the functions of the frontal lobes; degeneration of the spinal cord in cervical spondylosis; and poliomyelitis, on which latter subject he published a monograph, *Poliomyelitis* (1952).

Dr Ronald Bodley Scott was educated at Oxford University and at St Bartholomew's Hospital, London. He is a general physician with particular interest in haematology and is at present Assistant Physician to St Bartholomew's Hospital, where he was formerly First Assistant to the Medical Professorial Unit. He was Physician to the Royal Household from 1949 to 1952, when he was appointed Physician to H.M. The Queen. He has contributed numerous papers, chiefly on haematological subjects, to medical journals, amongst which are the following: "Sternal puncture in the diagnosis of diseases of the blood-forming organs" (*Quart. J. Med.* 1939, n.s. **8**, 127) and "The spleen and splenectomy" (*Brit. med. J.* 1949, **1**, 1063). He has also contributed chapters on the indications and complications of blood transfusion to *Blood transfusion* (1949), edited by Geoffrey Keynes.

Dr W. G. Spector is a Lecturer in the Department of Morbid Anatomy at University College Hospital Medical School, London, where he is engaged in research and teaching, and where he was formerly Graham Scholar in Pathology and a Beit Memorial Fellow. He is interested in the application of biochemical techniques to pathology and has published papers on renal disease, inflammation and electrolyte metabolism, and is now engaged in studying some aspects of proteinuria and of plasmaprotein metabolism. His other main interest is in stillbirths and neonatal mortality.

Dr H. B. Stoner began working on the reaction of the body to injury in 1943, at first as a member of the Biological Research Staff Pool of the War Office attached to the Department of Pathology of the University of Sheffield, and later as a member of No. 2 Traumatic Shock Team, Royal Army Medical Corps. Since the Second World War he has worked on this subject in the Department of Pathology at the University of Sheffield, and as a Fellow of the Rockefeller Foundation at Harvard University from 1948 to 1949. He has been a member of the Scientific Staff of the Medical Research Council since 1949 and is now a member of the Council's Toxicology Research Unit at Carshalton, Surrey, where he is concerned with the extension of his work to include the response to chemical injury. With Professor H. N. Green and others, he has published papers on the reaction to injury and the physiological behaviour of the adenine nucleotides, and a book entitled *Biological actions of the adenine nucleotides* (1950).

Professor R. H. S. Thompson has previously contributed two articles to the Bulletin, with both of which biographical notes were published. The first paper was on therapeutic applications of British anti-lewisite (BAL) (*Brit. med. Bull.* 1948, **5**, 319), and the second on cholinesterases (*Brit. med. Bull.* 1953, **9**, 138).

Professor G. Payling Wright is Sir William Dunn Professor of Pathology in the University of London and Honorary Pathologist to Guy's Hospital, London. Formerly, he was a Research Fellow at Harvard Medical School, where he worked with Professors E. J. Cohn and G. R. Minot, and Pathologist to University College Hospital, London. He has been interested in the epidemiology of infectious diseases, and was joint author with Dr P. D'Arcy Hart of a monograph entitled *Tuberculosis and social conditions in England, with special reference to young adults (a statistical study)* (1939). For some years he has been concerned with the application of physiological methods to the study of the modes of action of bacterial toxins, and has recorded observations in a number of papers in pathological and other medical journals. He is the author of a work on general pathology entitled *An introduction to pathology* (1950).

BODY-FLUID DISTRIBUTION IN INJURY

F. C. COURTICE M.A. D.Phil. D.Sc. L.R.C.P. M.R.C.S.

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1 Normal body-fluid distribution
  a Intracellular and extracellular phases
  b The capillary membrane
  c The role of the lymphatic system
  d Fluid exchange with the exterior
2 Fluid distribution in injury
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  b Reabsorption of fluid into the circulation
  c Capillary permeability in uninjured tissues
  d Fluid exchange with the exterior
3 Conclusion
References

An environment constant in composition and temperature is essential for the normal metabolism of the cells of the mammalian organism. Despite variations in the external environment, the many protective mechanisms ensure that the volume and composition of the extracellular fluid, which constitutes the immediate environment of the cells, are in normal circumstances not much altered. Should external violence of sufficient magnitude be encountered, however, local reactions may result in a disturbance of the fluid balance, causing swelling of the injured tissue, and these changes may in turn involve the organism as a whole. A general survey of these shifts in body fluid will be given here.

1. Normal Body-Fluid Distribution

The total water content of the adult human being is 50% to 60% of the body-weight (Edelman, Haley, Schloerb, Sheldon, Friis-Hansen, Stoll & Moore, 1952), but if allowance is made for fat, which contains but little water, it is about 71% (McCance & Widdowson, 1951).[1] This water acts as a solvent for the various solutes and is thought to be readily diffusible throughout the body (Peters, 1944). The osmotic pressure of the fluids within the body is, therefore, uniform. Though water itself is freely diffusible, there are barriers to the free movement of the electrolytes, so that the composition or ionic pattern of the body fluids varies in different compartments. There are at least two such barriers, the cell membrane and the capillary membrane.

a. Intracellular and Extracellular Phases

The cell membrane separates the water of the body into two phases, the intracellular and extracellular, the electrolyte pattern of each phase being completely different (Gamble, 1952). The extracellular fluid contains most of the sodium and in general the volume of extracellular fluid runs parallel with the total sodium content of the body. On the other hand, most of the potassium of the body is intracellular. Various theories have been postulated to explain the way in which the cell membrane acts as a barrier to these electrolytes. Whatever the mechanism, the maintenance of large electrolyte gradients between extracellular and intracellular fluids requires the liberation of considerable quantities of energy by the cells.

In the body as a whole the extracellular water is approximately 20% and the intracellular water 50% of the lean weight, but these two phases vary greatly in the different tissues. For example, of the two tissues of greatest bulk in the body, skin has a high extracellular and a low intracellular phase, whereas the reverse holds for muscle (Harrison, Darrow & Yannet, 1936). A relatively high proportion of the total extracellular water of the body is, therefore, present in the skin, while the greater part of the intracellular water resides in the muscles. In times of need the organism draws its water mainly from these two sources, with a resultant dehydration of the skin and wasting of the muscle (cf. Cameron & Courtice, 1948).

b. The Capillary Membrane

The second barrier, the capillary membrane, further divides the extracellular phase into two compartments: the intravascular, about 5%, and the extravascular, about 15% of the body-weight. This membrane maintains these two fluid volumes by acting as a barrier to the large plasma protein molecules. A constant exchange of water and dissolved substances takes place across the capillary membrane and the balance of fluid is maintained by physical forces. The capillary blood pressure tends to force fluid out of the capillaries into the extravascular space, opposed by the osmotic effect of the plasma proteins and to a certain extent by the hydrostatic pressure of the extravascular interstitial fluid.

c. The Role of the Lymphatic System

The capillary membrane is not, however, a complete barrier to the proteins, for most capillaries leak protein to a certain extent. Evidence suggests that, once these protein molecules become extravascular, they can be returned to the blood only by way of the lymphatics (Drinker, Field & Homans, 1934; Courtice & Simmonds, 1949a; Courtice & Steinbeck, 1950, 1951). The membrane of the lymphatic capillary is complete and forms a further potential barrier within the extracellular phase. Although the permeability of the lymphatic capillary membrane may vary (McMaster, 1942), in general protein molecules appear to enter the normal lymphatic capillaries very readily (cf. Drinker & Yoffey, 1941). The quantity of protein that can be obtained by collecting lymph from various regions of the body amounts to 50–100% of the circulating plasma protein per day (Courtice, Simmonds & Steinbeck, 1951), but it seems that the permeability of the capillary membrane to protein varies considerably in different regions of the body (Courtice, 1953).

d. Fluid Exchange with the Exterior

Apart from these fluid exchanges within the body, there is also an exchange of water between the body and the exterior. Through the skin and lungs there is an obligatory loss of water necessary for the regulation of body temperature. The total body water is, however, normally kept constant by the sensation of thirst regulating intake and by the kidneys adjusting

[1] See also McCance & Widdowson, *Brit. med. Bull.* 1951, 7, 297.—Ed.

output. Two important factors in the final adjustment of the body's water and sodium balance by the renal tubules and, therefore, in the maintenance of the extracellular fluid volume, are the antidiuretic hormone of the posterior pituitary gland and the hormones of the adrenal cortex (cf. Verney, 1947; Pickford, 1945; Gaunt, Birnie & Eversole, 1949).

2. Fluid Distribution in Injury

The disturbances in body-fluid distribution caused by injury mainly concern the extracellular phase. Injury may completely destroy a tissue, or, on the other hand, may only alter the effectiveness of the barriers that normally prevent the free movement of electrolytes or of proteins. The resultant disturbance of fluid distribution is first seen in the injured tissue, but secondary adjustments in the body as a whole then occur, which help to preserve the life of the organism by maintaining intravascular volume and blood pressure.

a. Local Loss of Fluid

A local accumulation of fluid in the extravascular spaces, leading to swelling of the damaged tissues, follows all injuries. The cells undergo changes which might result in a disturbance of the exchange of electrolytes and of other substances through the cell membrane (cf. Cannon & Bayliss, 1919; Leach, Peters & Rossiter, 1943; Cameron, 1952), but the damaged cells contribute directly very little fluid to the oedema. Indirectly, however, substances escaping from the injured cells are thought to be mainly responsible for the local loss of fluid by their action on the capillaries (Lewis, 1927).

The nature of the fluid loss depends upon the type and the intensity of the injury. In mechanical trauma the tissues may be crushed and lacerated with widespread rupture of the blood vessels, resulting in an effusion of blood into the neighbouring extravascular spaces. The blood vessels of less severely damaged tissue may not be ruptured, but the permeability of the capillary membrane to the plasma proteins is increased. The swelling of the damaged tissue in mechanical trauma is, therefore, due mainly to haemorrhage, but in part also to the effusion of a protein-rich fluid resembling plasma (Parsons & Phemister, 1930; Blalock, 1930, 1931; Freedlander & Lenhart, 1932). In those injuries such as thermal or chemical burns in which there is little or no rupture of the blood vessels, intense local oedema is due to failure of the capillary membrane to act as an efficient barrier to the protein molecules. The balance between hydrostatic and osmotic pressures is consequently disturbed, with exudation into the extravascular compartment. The protein concentration of this fluid is always high, approximating to that of plasma (Beard & Blalock, 1931; Harkins, 1945; Cameron & Courtice, 1946).

Since tissue fluid and lymph are of approximately the same composition (Drinker & Yoffey, 1941), the effects of various grades of injury on the protein concentration of the tissue fluid can be measured by collecting lymph from the affected region. The changes in capillary permeability that are caused by thermal injury can, for example, be readily determined in this way. When the temperature of a limb is raised the arterioles dilate, thus increasing the capillary pressure and blood flow through the region. This results in an increased formation of capillary filtrate and of tissue fluid, but with no significant alteration in capillary permeability to protein until the temperature exceeds 50° C. At about 55° C., however, the permeability alters, to allow easy passage of the protein molecules with a resultant intense oedema of the injured skin (Courtice, 1946). The protein molecules now escape through the capillary membrane as readily as do the small electrolytes (Cope & Moore, 1944). The lymph flow increases considerably as the pressure of the interstitial tissue fluid rises, and the protein concentration of the lymph rises until it approximates to that of the plasma. The osmotic effect of the plasma proteins is thus lost in these capillaries of the injured tissue. If, however, the temperature is excessively high, the tissues, including the blood vessels, will be coagulated or destroyed, so that no blood will flow through the area. For example, if a rabbit's ear is placed in water at 100° C. for half a minute, the whole ear will shrivel up with little or no oedema formation, whereas if the ear is placed in water at 65° C. for the same time, it will ultimately swell enormously.

In any injury, therefore, there will be zones in which the capillaries are affected in different ways—the zone in which the capillaries are necrosed or thrombosed and through which blood no longer flows, the zone in which the capillary membrane loses its effectiveness as a barrier to protein, and the zone in which the capillaries may be altered in calibre but are otherwise normal. The extent of each of these zones in an injury determines the extent of protein extravasation. In some instances, the first zone of tissue necrosis may be small, yet surrounded by a large zone of intense oedema. Such is the case with skin contamination of certain vesicant liquids (Cameron, Courtice & Short, 1947; Cameron & Courtice, 1948), and in certain injuries such as the experimental crush syndrome (Bywaters & Popjak, 1942; Swingle, Remington, Drill & Kleinberg, 1942; Duncan & Blalock, 1942).

The sudden shift of fluid in the injured region from the intravascular to the extravascular compartment decreases the intravascular volume and, if the extent of this fluid shift is sufficiently great, peripheral circulatory failure results. One of the most controversial issues in injury during the present century has been whether or not this primary local loss of fluid from the circulation is alone sufficient to cause the circulatory changes of "shock". Cannon & Bayliss (1919) concluded from their investigations in the First World War that in mechanical trauma the damaged muscles probably liberated a toxic metabolite which was the main cause of the circulatory changes in shock. Blalock (1931), however, showed that in dogs mechanical trauma to the intestines or to the extremities, or thermal burns of one side of the body, produced a local extravasation of blood or protein-rich fluid of the order of 4% to 5% of the body-weight. He compared the fall in blood pressure which occurred after mechanical trauma and thermal burns with that which followed repeated haemorrhages, so controlled that the blood loss was similar in amount and time-course to the fluid trapped in the tissues after mechanical trauma and burns. His results led him to conclude that the degree of fluid loss in these conditions was sufficient to cause the circulatory changes observed. In thermal burns involving the hind legs of the rabbit, Courtice (1946) showed that scalds below the knee of both legs resulted in an extravasation of fluid greater than the original plasma volume and a considerable fall in blood pressure. When the rate of local fluid loss was greatly decreased by keeping the injured legs cold after scalding, there was no fall in blood pressure nor other signs of peripheral circulatory failure. Similarly, when the amount of local fluid loss from the circulation was greatly decreased by the application of pressure bandages immediately after thermal burns or after prolonged

tourniquet constriction, the degree of shock and the mortality rate were greatly decreased or abolished (Courtice, 1946; Cameron, Allen, Coles & Rutland, 1945; Swingle *et al.* 1942; Duncan & Blalock, 1942). These experiments suggest that in injury the events leading up to peripheral circulatory failure are initiated by the extravasation of whole blood or of plasma protein into the injured area.

b. Reabsorption of Fluid into the Circulation

The local loss of fluid from the intravascular to the extravascular space may be greater than the entire plasma volume. In such circumstances the circulation of the blood can be maintained only by a compensatory reabsorption of interstitial fluid into the blood stream. This fluid shift, which occurs whatever the extent of the initial loss, is brought about in two ways: (i) by the reabsorption directly into the blood capillaries of non-protein fluid from the extravascular space of the uninjured tissues, and (ii) by the increased lymphatic flow from the injured region.

i. *Reabsorption from uninjured tissues.* At first the inward shift of fluid from the uninjured tissues is slower than the outward passage of fluid into the injured area, so that, if there is no red-cell loss, the blood will become concentrated. If, on the other hand, the loss of fluid is due mainly to haemorrhage, as in mechanical trauma, the blood may become diluted. When the loss of fluid from the injured area is speeded up by keeping the damaged part warm, the reabsorption of fluid is also speeded up and the plasma protein concentration consequently falls more rapidly (Courtice, 1946). The rate of this inward shift of non-protein fluid through the blood capillaries depends on the fall in capillary pressure which is determined by the rate and extent of fluid loss from the circulation in the injured area. The extent of the shift also depends on the available extracellular fluid in the undamaged tissues. The resultant fall in intravascular volume, and so the degree of shock, is greater if the organism has had previously a restricted intake of water. This is so because there is less available extracellular fluid (mainly in the skin) from which the inward shift of fluid is drawn (Cameron & Courtice, 1946).

After a certain time, about six hours in some injuries but longer in others, the relation between the outgoing and incoming fluids changes, and the haematocrit begins to fall (Courtice, 1946; Bywaters & Popjak, 1942). This is due, not to a healing of the damaged capillaries, but to increased hydrostatic pressure of the accumulated interstitial fluid which opposes the capillary pressure and so slows down the loss of fluid from the blood stream. The altered permeability of the capillaries does not return to normal for three to five days, as has been shown by releasing pressure bandages at various times after injury (Swingle *et al.* 1942; Cameron *et al.* 1945). When the demand for fluid to maintain the intravascular volume is prolonged, intracellular water is liberated, chiefly from the muscles, and is associated with loss of weight by these tissues and release of potassium and nitrogen (cf. Elkinton & Winkler, 1944).

ii. *Lymphatic drainage from injured tissues.* Since the extravascular fluid in the injured tissues is rich in protein, it is returned to the blood stream mainly by the lymphatics. Experiment has shown that soon after injury, when the tissue fluid formation in the damaged area is greatly increased and a large pool of " free " fluid is present in the tissues, the tissue tension is raised (McMaster, 1946). In these circumstances the flow of lymph from the injured area is very greatly increased. The lymphatics, therefore, are capable of increasing the protein absorption many times above normal, but, even so, gross oedema results. Later, when the extravasated fluid clots, the lymph flow decreases. A sudden decrease in lymph flow from an injured area in the acute phase may also be brought about by the injection of a tissue extract to coagulate the oedema fluid (Glenn, Peterson & Drinker, 1942).

When the capillaries regain their normal permeability in three to five days, one of the problems in the complete restoration of the fluid distribution in the injured tissue is the reabsorption of all the extravasated protein and of the fibrin clot. While the fibrin must of necessity be digested by fibrinolytic enzymes, there is evidence that the albumin and globulin fractions are absorbed by the lymphatics as such without being broken down to smaller molecules (Courtice & Simmonds, 1949b). This lymphatic absorption in the later stages of recovery is, however, much slower than in the acute phase, partly because of the formation of the fibrin clot and partly because the tissue tension is not as great as in the initial phase when the connective tissue fibres are suddenly stretched.

c. Capillary Permeability in Uninjured Tissues

Although as a rule the passage of water through the capillaries of the uninjured tissues, especially in the early stages, is mainly inward to compensate for the loss of fluid in the injured region, much controversy has arisen concerning the permeability of these capillaries. This might be affected by the lowered oxygen tension resulting from the peripheral circulatory failure or by a toxic substance liberated from the injured tissue or from elsewhere. Landis (1928) has shown that severe anoxia can affect the permeability of the capillary membrane to the plasma proteins, but Courtice & Korner (1952) concluded that moderate anoxia in unanaesthetized rabbits breathing 10% oxygen did not significantly affect the permeability of the pulmonary capillaries to protein. Korner & Courtice (unpublished data, 1953), by determining the extravascular circulation of protein from various regions of the cat, have also shown that breathing 10% oxygen has no appreciable effect on the permeability of the capillaries to protein. With regard to the liberation of a toxic substance from the damaged tissues, a great many hypotheses have been postulated (cf. Harkins, 1941), but, as yet, no one has shown with certainty that any substance is liberated in amounts sufficient to increase significantly the permeability of the capillaries in the undamaged tissues.

The efficiency of the capillaries of the undamaged tissues as barriers to the plasma proteins has actually been determined after injury by two methods. By collection of lymph from uninjured areas, it has been shown that the extravascular circulation of protein in these areas has not been increased in animals suffering from severe shock resulting from thermal or chemical injuries (Glenn, Muus & Drinker, 1943; Cameron *et al.* 1947; Courtice, unpublished data). In other experiments labelled plasma proteins have been injected intravenously into animals suffering from severe shock caused by haemorrhage, thermal burns or mechanical trauma (Fine & Seligman, 1943, 1944; Cope & Moore, 1944). These experiments show that, as a rule, there is no significant increase in the permeability of the capillaries to protein other than in the injured area.

d. Fluid Exchange with the Exterior

After a severe injury the urinary output is decreased, partly because of the altered haemodynamics (fall of blood pressure and vasoconstriction of the renal vessels) decreasing the glomerular filtration, and partly because of the increased concentration in the circulation of the antidiuretic hormone from the posterior pituitary, which increases the reabsorption of water by the tubules. Stimulation of the posterior pituitary is probably caused more by emotion and by afferent impulses from the traumatized region than by a rise in the osmotic pressure of the plasma (Le Quesne & Lewis, 1953). In surgical trauma it has been shown that, in addition to water retention, there is a retention of sodium, associated with the other signs of increased activity of the adrenal cortex (Moore, 1953). These effects of the adrenocortical and post-pituitary hormones in injury help to maintain the extracellular fluid volume and thereby support the plasma volume and blood pressure.

3. Conclusion

In injury the alterations in fluid distribution of the body are concerned mainly with the extracellular phase, at least in the early stages. The primary shift of fluid is from the intravascular to the extravascular space in the injured tissues. Although the lymph flow from this region is greatly increased to return the extravasated fluid to the circulation, the main compensatory fluid shift is from the extravascular to the intravascular space of the uninjured tissues. To maintain the extracellular fluid volume, increased activity of the posterior pituitary and adrenal cortex aid water and sodium retention in the body, and water is provided from the cells, chiefly by the breakdown of muscle cells. The aim of these compensatory shifts in fluid is primarily to maintain the intravascular volume necessary for a normal blood pressure and for adequate circulation to the vital tissues of the organism.

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SHOCK CAUSED BY BURNS AND ITS TREATMENT

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1 Pathogenesis
2 Treatment
3 Results of treatment
References

The general bodily disturbance caused by severe burns is similar to the "shock" due to oligaemia from acute haemorrhage, but there are sufficient important differences to require separate study. Even in respect of the similarities, the timing and severity of various changes are different and there are further features in burns not paralleled in other forms of trauma.

The importance of timing is evident when it is realized that a patient with perhaps 40% of the surface area of the body burned can be admitted to hospital one or two hours later showing very little systemic disturbance. Ten hours after this the patient may be in severe and possibly irreversible shock if nothing has been done in the meantime to anticipate these changes. The leak of plasma from a burn is a gradual process and at first the body can adapt itself to the loss by redistribution of fluid and vasoconstriction, but, if the leak continues without replacement, the diminished circulatory volume produces the classical signs of lowered blood pressure, cold skin, raised pulse rate and ultimately death.

1. Pathogenesis

There is general agreement that the main cause of burns shock is oligaemia, chiefly due to loss of plasma. To the external loss into blisters and from open surfaces is added the oedema of tissues deep to and surrounding those destroyed. Animal experiments show that a burn of half the body surface may cause a local fluid loss into the tissues about equal to the plasma volume (Rossiter, 1943). Added to the local swelling is the volume of the greatly increased flow in the lymphatics draining the area (Cope, Graham, Mixter & Ball, 1949). To what extent there may be vasodilatation in other parts of the circulation at this early stage is not certain, but it is a striking finding, at post-mortem examination made at a rather later period, that many visceral vessels are grossly congested, and some experiments suggest that this is also important at an earlier stage (Prinzmetal & Bergman, 1945). The fluid which leaks from the damaged capillaries consists of a rather dilute plasma. It contains all the plasma proteins in about the same proportions as they occur in the general circulation and also contains the normal electrolyte complement of plasma. It is sometimes assumed that the exudate is as dilute as half-strength plasma in respect of proteins, but estimates of the protein content of blister fluid and exudate fluid frequently show levels almost as high as those currently present in the circulation. If in therapy a plasma substitute such as dextran or polyvinylpyrrolidone is given, this substance will also appear in the exudate.

Further to the electrolyte changes consequent upon this physical leak, are the exchanges between intracellular and extracellular fluid due to cellular damage. In general, damaged cells fail to maintain their differential concentration of potassium and sodium as compared with the extracellular fluid, and as a result sodium enters the cells and potassium leaves them. The probable extent of this change in the burned area has been studied by Moore, Evans & Ball (1948) and it would appear to be a relatively minor factor in the total electrolyte exchange. There is evidence that very severe and rapidly induced burns, such as might occur near an atomic explosion, do result in dangerously high levels of serum potassium from the sudden release by damaged cells but, in the usual civilian case of burns, toxic levels of potassium are probably seldom reached. The extent of sodium loss into the cells is still uncertain. A feature of treated burns is that, in spite of administration of large quantities of sodium, the serum sodium level tends to fall during the shock period. Whether this is evidence of continued deficiency or is in some way an inevitable response to burning is uncertain. It has been claimed that a high sodium input is all-important in the treatment of burns shock, but validation of this in human patients is incomplete.

Whatever the fate in the body of administered sodium and water, there is, with present methods of treatment, a very marked retention of these substances by the kidney. This retention commences very soon after burning and continues for several days. During this period it is possible to raise the excretion of sodium and water to some extent by giving further quantities, but only a very small proportion is excreted and the resulting marked positive balances and greatly expanded extracellular fluid volume are thought to be dangerous.

As a result of tissue breakdown there is a tendency to acidosis during burns shock and this is accentuated by tissue anoxaemia if circulatory volume is inadequately replaced. It is further increased if saline is used as a replacement fluid, since not all the sodium in extracellular fluid is balanced by chloride and the administration of the extra chloride tends to displace bicarbonate. In practice these tendencies towards acidosis in treated cases are frequently more than offset by the administration of plasma or of whole blood. Both these contain added sodium citrate and have a strong alkalinizing effect, often causing the urinary pH to reach very high levels. Since dextran, which is made up in saline and therefore has an acidifying effect, gives clinical results very similar to those of plasma, it seems unlikely that pH disturbance during burns shock is of great practical importance.

Studies of the local vascular changes in the burned area show dilated vessels through which the blood flows sluggishly, and which often become filled with masses of static red blood cells (Sevitt, 1949). These red cells are lost to the active circulation and it is probable that other red cells that have passed through the burn become abnormally fragile and are soon haemolysed. These losses of red cells contribute to the loss of circulatory volume and a few days after burning there

may be a red-cell deficit of 30–40%. How much red-cell destruction or sequestration occurs during the shock phase is still the subject of research. Haemoglobinaemia and haemoglobinuria are present in some severe burns but not in all, and it may be that different speeds, temperatures and depths of burning cause different amounts of red-cell loss. There is a temporal sequence of vascular change in the region of the burn: vessels initially patent with active flow become blocked after perhaps 24 hours; if such changes occur in a large burn there must be corresponding fluctuations in the circulatory volume and in the relative amounts of red cells and plasma.

The extent to which changes during burns shock are mediated by chemical substances is uncertain. For the past 50 years or more, claims have been made that specific toxins are important. These have ranged from pyridine compounds, products of nuclear breakdown and peptones, to more recent suggestions of histamine, leucotaxin and adenosine triphosphate. It is very likely that a chemical agent is concerned in some way in the local vascular dilatation and increase of permeability. It is less certain whether circulation of such material in the blood plays an important part. Many of the animal experiments that are claimed to have demonstrated these substances have been thrown into doubt by other experiments; for example, the early work of Robertson & Boyd (1924) that was claimed to show toxic agents in burned tissues can be compared with the demonstration by Underhill & Kapsinow (1931) of toxic effects from unburned tissues equivalent to those after burning. A criticism of many of the experiments has been that they concerned untreated burns, and it may well be that the agents causing shock and death in the untreated animal are different from those present in the treated human case. A further source of confusion has been the difficulty of excluding bacterial toxins. Most large burns in the past, and many still in the present, become infected. The raw area of the burn freely absorbs soluble materials and this must include bacterial toxins in infected cases. That the presence of the raw area is in some way noxious is evident from the clinical improvement that follows successful grafting. Some of this effect may be due to absorbed toxic breakdown products, some to bacterial toxins; but it must also be remembered that the effect might be due to loss of important substances from the burned area. It is known that important amounts of protein are so lost, and there may be some factor as yet unidentified that becomes deficient by leakage from the wound.

2. Treatment

The best treatment of burns shock is its prevention. This requires recognition of the cases which will develop shock, and the best guide to this diagnosis is the extent of the burn as measured in percentage of the body surface area. The estimation of area can readily be made with the aid of diagrams of the body surface upon which the outline of the injury can be charted. Several versions of the diagrams are available (Berkow, 1924; Lund & Browder, 1944). A convenient approximation is given by the " Rules of Nine " suggested by Wallace (1951). Each arm and the head and neck are approximately 9% of the surface area of the body, and each leg and the front and back of the trunk are each 18%. The perineum and genitalia bring the total to 100%. Only areas of moderate or severe burning should be included in making the estimate; so far as possible, areas consisting only of erythema should be excluded. Children with burns of 10% or more of the surface area have an appreciable risk of becoming severely shocked and should receive intravenous therapy; patients with less than 10% of the surface area burned can almost always be treated with oral fluids. In adults a critical area of 15% is usually safe, and even areas up to 20% can sometimes be managed satisfactorily with oral therapy. If repeated haematocrit observations are made on even these border-line cases, a period of haemoconcentration will be found. This presumably corresponds to a phase of diminished circulatory volume, apparently within the adaptive powers of the circulation.

The aim in the management of fluids is to maintain an adequate circulatory volume with the minimum of other disturbance. Since the circulation has a leak at the site of the burn, its volume can be maintained only by keeping pace with this leak. Even transfused plasma will leak out at the burn, but elsewhere in the circulation it will be retained. If saline is used it will be rapidly lost even into unburned tissues and will increase the risk of pulmonary oedema. Water alone is particularly dangerous, since it will dilute the body electrolytes and cause water intoxication (Moyer, 1949).

Burns of area greater than the critical levels are best treated by transfusion with plasma or its equivalent. The first thing to be done in such cases is to set up an intravenous drip, and this takes priority over any other treatment. The local care of the burns should be limited to covering with sterile cloths or, if preferred, exposure treatment may be commenced. The amount and rate of plasma administration can be regulated by repeated haematocrit estimations. It is important that the blood sample for the haematocrit be taken from a vein or capillaries of warm skin, so that the result will be representative of the general circulation. Since the initial haematocrit value may be already raised by haemoconcentration, the result must be compared with the appropriate normal value for the age of the patient. At birth the haematocrit value is high but, after the first month and up to five years of age, its normal value is about 38; it then rises gradually to the normal adult value of about 45 at 20 years of age. An alternative to the haematocrit estimation is an accurate haemoglobin estimation; when corrected for age, this is equally good, but since it is important that the doctor should be able to make his own estimations at any hour of the day or night, there is a considerable practical advantage in using a simple haematocrit technique which gives a reliable answer within 20 minutes rather than relying upon ward haemoglobin estimations which have a high margin of error. Whichever index of haemoconcentration is used, the estimations need to be repeated at about hourly intervals so that the rate of the continuous drip can be regulated to achieve and maintain a normal value and hence an approximately normal circulatory volume. In view of the known loss of red cells, the volume obtained by correcting to the normal haematocrit is probably rather less than the normal blood volume. During the transfusion it may be desired to give whole blood to anticipate the anaemia which follows severe burns. If this is done a new " normal " haematocrit can be calculated on the basis of the amount of red cells transfused. The haematocrit will rise in the proportion that the added red cells bear to the patient's previous red-cell volume. When the blood has been given the plasma transfusion can be continued and adjusted in rate to maintain the new " normal " haematocrit.

As an alternative to measures of haemoconcentration such as the haematocrit and haemoglobin, some authorities prefer to use blood-pressure measurements. With experience this may be satisfactory, but there is evidence that fall in blood pressure is a relatively late sign and that the earlier sign of haemoconcentration is a more sensitive guide. Another objection to the use of blood pressure is that it is frequently physically difficult to measure, since many severe burns involve the areas on the limbs most convenient for blood-pressure determination. Even if not used as a primary guide to the adequacy of the replacement of fluid, blood-pressure measurements, where they can be performed, are a useful additional source of information. Further to these estimates, the general condition of the patient must be carefully watched; thirst and restlessness, for example, suggest the need for increased fluid replacement. It should be remembered that during this stage symptoms and signs may be obscured by the action of morphine, which may have been administered subcutaneously much earlier, but now perhaps takes effect due to the return of circulation.

While plasma is being given intravenously, frequent small quantities of fluids should be given by mouth, sufficient to supply the normal requirements for urinary and extrarenal losses, with an extra allowance if there is any fever. For children total oral fluids of about 2 l. per day are satisfactory; in adults 3–4 l. may be necessary. When "border-line" shock cases are being treated by oral fluids only, the equivalent of about half-strength saline should be used. Sodium lactate (Fox, 1944) or a mixture of sodium chloride and sodium bicarbonate (Moyer, 1949) are alternatives to sodium chloride itself and are usually acceptable when given in sweetened fruit drinks. How much extra salt is needed when adequate transfusion is given is not certain. Reconstituted plasma and bottled blood both provide a protein level similar to that in burns exudate and with this protein there is rather more sodium (about 165 m-equiv. per l.) than there is in extracellular fluid. No great electrolyte disturbances follow the use of either no added oral sodium or a moderate addition. If vomiting is caused by salt, then salt-free oral fluid should be given. If vomiting persists, glucose or glucose-saline solution should be given intravenously.

The urinary output is a valuable index of the adequacy of resuscitation. To follow it hourly an indwelling catheter should be used for the first 48 hours after therapy is begun. If the urine flow is 30 ml. or more per hour, fluid management is probably satisfactory; if the flow is less than this, management should be reviewed to see whether perhaps insufficient fluid is being given, but it should be remembered that a period of oliguria down to about 20 ml. per hour is a common occurrence in severe burns and is quite consistent with recovery.

Review of cases treated by these methods shows that most satisfactorily managed cases receive half their plasma requirement in the first 8 hours or so, and the second half in the subsequent 16–24 hours. Similarly it is found that the total amount of plasma or its equivalent is approximately related to the size of the burn. In an adult each 10% of the surface area burned requires about 1 l. of plasma, in children the corresponding figure is a volume about equal to the patient's normal plasma volume per 15% of surface area burned; several formulae have been devised to assist in calculating the fluid replacement required for different sizes of burn. Some of these formulae allow of correction for the weight of the patient and provide approximately 1 ml. of plasma for each 1% of burning per kilogram of body-weight. Some of the formulae provide for administration of saline extra to this. Oral fluids are recommended at a rate of 25–50 ml. per kilogram of body-weight per day (Cope & Moore, 1947; Wallace, 1951; Evans, Purnell, Robinett, Batchelor & Martin, 1952). Rigid adherence to a formula is liable to result in unsatisfactory treatment of some cases, since surface area is not a completely adequate measure of fluid requirements. The formulae fit well with average requirements and are a useful check and guide to correct therapy, but each patient should be treated on his own merits, and haematocrit and haemoglobin readings are probably the best hour-by-hour indication of adequate correction of circulatory volume.

The use of plasma and—to a less extent—blood, carries with it the risk of jaundice. This risk is not usually very great; recent studies in Great Britain show an incidence of about 2% among patients receiving plasma, and about 0.8% among those receiving blood (Maycock, 1952). In some circumstances the risk may be much higher, and the jaundice can be of a severe type with a high mortality. Both reconstituted plasma and bottled blood have the further possible disadvantage of a high potassium content, up to 15 m-equiv. per l., and a considerable citrate content which may be harmful when large volumes are given rapidly. These considerations make it reasonable to examine alternatives to plasma in the treatment of burns. Of the currently available plasma substitutes[1], dextran and polyvinylpyrrolidone are the most eligible, and of these dextran, which provides carbohydrate molecules sufficiently large to be held in the circulation and so maintain the circulatory volume throughout the shock period, is probably preferable. Such dextran solutions have molecules of the approximate size of plasma proteins and the range of molecular size is limited so that only a small proportion is rapidly excreted and the remainder is not too large to be inefficient as an osmotic agent. When used in the treatment of burns, dextran can be given at the same rate and in the same quantities as plasma and it appears to have an identical effect in correcting haemoconcentration. The dextran molecules replace protein in the total circulating colloid, but this does not appear to have harmful effects so long as the amount of dextran given does not greatly exceed the patient's plasma volume. When given in such amounts, plasma-dextran levels of 2–2.5 g./100 ml. are commonly found in the serum, with corresponding protein levels of about 4 g./100 ml. It is probably preferable, if more transfusion be required, to continue with plasma or whole blood and so avoid excessive depression of the plasma protein level. A practical consideration is that, since dextran raises the erythrocyte sedimentation rate, it is liable to cause difficulty in cross-matching of the patient's blood. The patient's cells after receiving dextran can still be typed after washing, but if there is a dextran level of about 1 g./100 ml. or more in the serum, rouleaux formation makes cross-matching difficult. The best precaution is to take a sample of the patient's blood for cross-matching prior to giving the dextran.

During shock treatment it is important to anticipate the risk of infection. This is best done by avoiding as far as possible any contact of unsterile materials with the burn, by the wearing of masks and gowns by attendants, and by the administration of local and systemic antibiotics. Penicillin is probably the treatment of choice. Since *Pseudomonas*

[1] See Maycock, p. 29 of this number of the Bulletin.—Ed.

pyocyanea infection is particularly liable to occur in large burns, the use of prophylactic local polymyxin is a reasonable precaution (Jackson, Lowbury & Topley, 1951). Oxygen therapy appears to be of some value in certain cases, but no appreciable improvement occurs in most. Compared with the importance of transfusion and the prevention of infection, other ancillary features of treatment of burns shock play but a minor role. Moderate sedation with morphine is helpful; ¼ grain[2] initially given intravenously is usually adequate. There is a danger of overdosage if the drug is given subcutaneously, possibly in repeated doses by different attendants, and the effects of this may be to cause serious depression of respiration when the circulation is recovering during transfusion.

[2] 1 grain = 65 mg.—Ed.

3. Results of Treatment

The over-all prognosis of burns has been greatly improved since adequate transfusion treatment has been practised (Bull & Squire, 1949). With such regimes of treatment as those outlined, most cases of severe burns can now be carried through the shock phase, but there remain patients who now die later, perhaps at the second or third week, and we cannot be sure if some feature of the present treatment of shock may be inadequate or perhaps harmful, and so play a part in these delayed deaths. Infection very commonly is involved, and should be amenable to prophylaxis and treatment, and there are commonly profound metabolic changes. It seems likely that further advances in understanding and control of this later period should further greatly improve the outlook for patients with severe burns.

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REMARKS ON THE DIAGNOSIS AND TREATMENT OF "WOUND SHOCK" IN LIMB INJURIES*

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1 The problem of wound shock
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The following remarks are based on clinical observations made on injured patients during the Second World War; a full account has already been published (Grant & Reeve, 1951).

1. The Problem of Wound Shock

The initial object of these observations was to learn to recognize and treat the condition known as traumatic or wound shock. It was found that the term "wound shock" is loosely used. It is used to denote the pathological state or states responsible for the circulatory failure following injury. It is also and more commonly used to denote the symptoms and signs of that failure. Even in the second sense, there is no general agreement as to what signs and symptoms are required to justify a diagnosis of "shock". The term is sometimes restricted to the syndrome of pale face, cold hands, fast pulse and low blood pressure. It commonly implies no more than that the patient is pale and cold, and so seems ill, irrespective of pulse rate and blood pressure.

This loose usage causes confusion for three reasons. First, "shock" may mean one thing to one man and something else to another. Second, a number of diverse circulatory states that have pallor as a common feature may result from injury; but they differ in origin, present otherwise different clinical patterns and require different treatments. Third, under the term "shock" other conditions are sometimes included, in which, though blood pressure is low and the pulse rate fast, the face is not pale nor are the extremities cold.

It follows that the term "shock" as commonly used conveys no precise diagnosis and indicates no particular treatment. Therefore, in handling the injured patient, the medical officer should not rest content with applying this label, often after no more than a cursory examination, but should proceed to define more closely the disturbed state of the circulation and so determine the factor or factors responsible for the disturbance.

It is not always easy, or even possible, to determine precisely the factors responsible, because the circulatory disturbances following injury are extremely complex, owing to the variety of factors that may be involved. The following are examples of these factors. (i) An important—and in many injuries the chief—factor is blood loss. Loss of blood may be caused in different ways. Thus, injuries by physical violence cause blood loss by haemorrhage, those by burning and prolonged compression cause blood loss by the escape of plasma from damaged capillaries. Each form of blood loss has its special features and requires different treatment. (ii) The circulation may be disturbed by the sensory and emotional stimuli associated with the injury (e.g., pain and apprehension), by the temperature of the surrounding air, by the various infections liable to develop (e.g., peritonitis, gas gangrene) and by the treatment administered. (iii) Other factors disturbing the circulation are introduced by injuries to brain, spinal cord and lungs.

At any stage of the illness following injury, several factors may be acting together on the patient, though one or other may predominate at different times. The task of interpreting the observed clinical state in terms of the factors causing it is further complicated by the facts that (i) the same factor (e.g., pain) may give rise to different clinical patterns, the vasovagal and the hypertensive, and (ii) different factors (e.g., haemorrhage and infection) may give rise to the same circulatory pattern of cold hypotension (see sections 3 and 4).

Nevertheless, in spite of this complexity, it is usually possible for the medical officer at the bedside, by paying attention to a few symptoms and signs, to gain information adequate for assessing the patient's state and for guiding treatment. Since the proper treatment of the severely injured brooks no delay, and since often large numbers have to be dealt with in a short time, it is important that the symptoms and signs used for diagnosis should be as objective, simple and few as possible. It is not feasible here to do more than indicate briefly how this may be done in the early hours following one type of injury, that is, limb injuries, the most common of all. Great importance is attached to diagnosis and treatment in these early hours, for if then the patient's state is correctly assessed and the proper treatment carried out, much of the illness occurring during and after operation should be avoided.

2. Diagnosis

The war-time studies made it abundantly clear that by far the most important factor disturbing the circulation in patients with limb injuries is haemorrhage, and that transfusion of whole blood is the most effective remedy. It is of first importance therefore to be able to assess blood loss so that adequate transfusion may be given. For this purpose, two main indices are used: the size of the wound and the systolic blood pressure.

3. The Factor of Haemorrhage

Measurements of blood volume show that, in general, the larger the wound, the greater the loss of blood, and that a clinical estimate of the size of the wound, in terms of volume of tissue damaged, affords a valuable index of blood loss.

* Work undertaken on behalf of the Medical Research Council.

TABLE I. CIRCULATORY PATTERNS SEEN IN WOUND SHOCK

	Normal	Cold tachycardia	Warm tachycardia	Hypertension	Vasovagal	Cold hypotension	Warm hypotension
Blood pressure*	Normal	Normal	Normal	Raised	Low	Low	Low
Pulse rate†	Normal	Fast	Fast and bounding	Normal or slow	Slow	Fast	Fast
Extremity temperature	Warm	Cold	Warm	Warm or cold	Cold	Cold	Warm
Face colour	Good	Pale	Usually well coloured	Well coloured or pale	Pale	Pale	Well coloured or pale

*Blood pressure
Raised: over 140 mm. Hg
Normal: 100–140 mm. Hg
Low: under 100 mm. Hg

†Pulse rate
Fast: 100 or more/min.
Normal: 70–99/min.
Slow: under 70/min.

The volume of tissue damaged is judged by inspection of the wounds, taking into account such obvious signs as abrasion, bruising, tearing and destruction of the soft parts, loss of contractility of muscles and fractures of bones. As a guide to the volume of damaged tissue, we take as the unit the patient's hand. The open hand may be used to assess the damage in superficial wounds, and the closed hand to estimate the volume of a bruised or pulped segment. In a man of average size the volume of the hand, open or closed, is just under a half litre. The foot, knee, forearm and upper arm are approximately equal in volume (the foot and knee each rather less, and the upper arm rather more, than the forearm) and each is roughly equal to two or three times the volume of the hand. Leg volume is four to five times and thigh volume ten to twelve times that of the hand. The injuries are grouped under four categories according to size in terms of the volume of tissue judged damaged: (i) small wounds, with damage of less than one hand; (ii) moderate wounds, with damage of one hand or more but less than three hands; (iii) large wounds, with damage of three or more but less than five hands; (iv) very large wounds, with damage of five hands or more in volume. In general, patients with small wounds rarely lose more than 20% and commonly less than 10% of their blood. The blood volume of a male of average size may be taken as 5 l. or approximately 10 pints. With moderate wounds, blood loss in the majority of cases is of the order of 20% to 40%. With large wounds much blood is lost, of the order of 40%, and with very large wounds, very much, of the order of 50%.[1]

The second valuable index is the level of systolic blood pressure, for this shows how far blood loss has reduced the blood volume. In general, it may be concluded that (i) if the blood pressure is at least 100 mm. Hg, the blood volume is not less than 70% of normal, and (ii) if the blood pressure is below 100 mm. Hg, blood volume is less than 70% of normal.

These two indices alone usually leave little doubt about the order of blood loss and the blood volume, and their evidence is usually concordant. Small and moderate wounds are usually associated with normal blood pressures, a combination which makes it reasonably certain that blood loss has been insufficient to reduce the blood volume to below 70% of normal. Large wounds are usually, and very large wounds are almost always, associated with low blood pressure, and the combination indicates a blood volume below 70% of normal; the larger the wounds the further below this level the blood volume is likely to be.

Further indication of how far haemorrhage has reduced blood volume may be provided by the subsidiary features of pulse rate, colour of face and temperature of extremities. Each of these by itself is of little value; but their combinations with each other and with blood pressure provide circulatory patterns of diagnostic value. These are summarized in Table I.

i. *Normal circulatory pattern.* This is a systolic blood pressure of 100 mm. Hg or more, associated with a pulse rate between 70 and 100 per minute, warm extremities and a good facial colour, which indicates that in all probability the blood volume is nearly normal, probably at least 90% of normal.

ii. *Pattern of cold tachycardia.* A blood pressure of 100 mm. Hg or more, associated with a pulse rate of 100 or more per minute, cold extremities and a pale face, indicates that the blood volume is probably between 70% and 80% of normal.

iii. *Pattern of cold hypotension.* A combination of blood pressure below 100 mm. Hg with a pulse rate of 100 or more per minute, cold extremities and pallor, is a clear indication of blood loss sufficient to reduce the blood volume below 70% of normal. This pattern tends to persist until abolished by transfusion. In patients with a great blood loss and a blood volume reduced below 60% of normal, an extreme form of this pattern develops, that is, a very low blood pressure (under 70 mm. Hg), impalpable pulses, a very rapid heart rate (140 or more per minute), cold extremities with constricted veins, and pale face and lips. To these may be added great restlessness, dyspnoea, and sweating. This form indicates the need for immediate, large and rapid transfusion to save life.

4. Complicating Factors

It is necessary to recognize, however, that other factors may disturb the normal circulatory pattern in the absence of loss of blood and may modify the effects of haemorrhage. Examples of these are as follows.

1. *Nervous and emotional stimuli*, such as pain, fear and apprehension, may provoke either of two distinct clinical patterns:

i. *Hypertensive pattern.* In this, blood pressure is raised, to over 140 mm. Hg; usually the pulse rate is normal or slow; the radial pulse may be barely palpable. The extremities may be warm or cold and the face pale or well coloured. This pattern is associated with little or

[1] See Scott, Table I, p. 23 of this number of the Bulletin.—Ed.

no blood loss; blood volume, if reduced, is not below 80% of normal. It is common soon after injury and is usually transient. In exceptional cases it may persist for a day or two.

ii. *Vasovagal pattern* is characterized by a blood pressure under 100 mm. Hg, a slow pulse rate (under 70 per minute), cold extremities and a pale face. To these may be added yawning or sighing respirations, sweating, nausea and vomiting. It is common soon after injury, even after trivial injuries, and is usually transient. Occasionally it is associated with much blood loss and is then apt to be prolonged. It may be seen as a terminal pattern in patients dying from haemorrhage.

2. *Temperature of the surrounding air.* Exposure to cold cools the extremities, slows the pulse rate and slightly raises blood pressure. Cold, by increasing the peripheral vasoconstriction associated with haemorrhage, increases the difficulty of inserting a needle into a vein for blood sampling or transfusion. Warmth, on the other hand, heightens facial colour, warms the extremities, increases pulse rate and slightly lowers blood pressure. Undue warming associated with haemorrhage may provoke the *pattern of warm hypotension*, that is, a blood pressure under 100 mm. Hg, a pulse rate of 100 or more, and warm extremities; the face may be well coloured or pale. It may be noted that this pattern is also to be met with during and after operation, when it is thought to be due to a combination of factors such as the anaesthetic agent, body warming and a previous transfusion. This pattern is usually, but not always, transient. Blood volume is generally reduced, although not below 70% of normal.

3. *Infection.* If the patient is not seen till eight or more hours after injury and heavy infection has developed, this, by poisoning the control of the circulation, may lead to the development of the pattern of *cold hypotension*, although blood volume may be normal. When infection is the cause of this pattern, the pulse rate tends to be more rapid and the vasoconstriction less than when haemorrhage is the provoking factor. Circulatory failure due to infection, unlike that due to haemorrhage, does not usually respond to transfusion.

4. *Transfusion reaction.* If the patient has been given a transfusion of blood before being seen he may have suffered a febrile transfusion reaction. This reaction starts with a phase of vasoconstriction, which is followed sooner or later by a phase of vasodilatation. During the constrictor phase, the circulatory pattern is that of *cold tachycardia* already described, even though blood volume may be normal. During the dilator phase, the circulatory pattern is that of *warm tachycardia*, a blood pressure of 100 mm. Hg or more, a fast and bounding pulse, warm extremities and a face usually well coloured, but sometimes pale. If blood volume is reduced, it is not below 70% of normal. It may be noted that this pattern is also met with in the late stages of the reaction to haemorrhage, when blood volume has been restored to about normal but haemoglobin has fallen to very low levels.

5. Treatment

At the beginning of the Second World War, there were no satisfactory answers to a number of practical questions. These questions were: which patients should receive transfusion; what transfusion fluid should be given, and when, at what rate, and how much; and when should operation be undertaken. The experience of the war years provided clear answers.

a. Patients to be Given Transfusion

Those patients do best, before, during and after operation, whose blood volumes are not far from normal, and well above the critical level of 70%. Hence, all patients whose wounds and circulatory state make it likely that the blood volume is below or not far above this level should receive transfusion. If in doubt, transfuse.

b. Fluid to be Transfused

Since loss of whole blood is the chief factor causing circulatory disturbance, blood is the best fluid to transfuse, provided its quality can be relied on. Circumstances arise in which sufficient blood is not available for transfusion. However, blood volume can be raised and the blood pressure restored by plasma or serum or a plasma substitute[2], even after gross loss of blood. But in such cases the patient remains seriously deficient of red blood cells. It is probable that a patient with a low red-cell volume is less able to supply oxygen to his tissues, to carry out repair and to prevent or overcome infection than one well supplied in this respect. The deficit should be remedied as soon as possible by transfusion of red cells.

c. Time of Transfusion

Though the circulation can be restored in many cases of hypotension, even though transfusion is delayed for an hour or two, delay is unwise. If a patient shows the extreme form of cold hypotension already noted, there must be no delay in beginning transfusion, if life is to be saved.

d. Rate of Transfusion

Slow transfusion not only delays recovery but, in patients with marked circulatory failure, it may result in early death. In such cases, transfusion must be at the rate of a pint[3] bottle of fluid given over a period of 5 to 15 minutes. When the patient has responded well to transfusion, its rate should be slowed to a pint bottle over half an hour or longer. In less serious cases and where transfusion is given prophylactically, the slower rate is sufficient, provided that the patient receives an adequate amount before proceeding to operation.

e. Amount of Transfusion

It is commonly thought that restoration of the blood pressure to 100 mm. Hg or above indicates that an adequate amount has been transfused. But this index is unsatisfactory and, in patients so treated, the circulation is very liable to fail again during operation. Restoration of blood pressure does not mean that blood volume has been restored to normal, but usually only that it has been raised to 70% of normal or above. If, however, transfusion not only raises blood pressure to normal but also slows the pulse rate and replaces vasoconstriction by vasodilatation, then blood volume has probably been raised to not far short of normal. Transfusion, and rapid transfusion specially, does not often do this because of the frequency of transfusion reactions. These, as has been pointed out, disturb the circulation, and so the features of pulse rate, face colour and extremity temperature do not often help to determine whether or not transfusion has been adequate. The most generally useful rule is to restore blood pressure to normal levels, which indicates that blood volume has been restored to about 70% of normal, and to continue

[2] See Maycock, p. 29 of this number of the Bulletin.—ED.
[3] 1 pint = 0.568 l.—ED.

transfusion beyond this point with a further amount, depending on the level of blood volume it is desired to reach.

It is not necessary to restore blood volume to normal, since patients can pass through operation safely with a blood volume of about 80% of normal. Moreover, there is some risk attached to the transfusion of stored blood and its products. It would seem advisable, therefore, to transfuse as little as is compatible with safety at operation. If one allows a margin for renewed bleeding at operation and for continued oozing afterwards, 90% of normal seems a safe level of blood volume to aim at for the beginning of operation.

As has been seen, the estimation of the size of the wound and the examination of the circulatory state before transfusion provide an index to the level of blood volume. Other observations have shown that a pint bottle of plasma, blood or serum (making allowance for the added diluents) may be expected to raise the blood volume by approximately 5%, 8% or 9% respectively of the predicted normal blood volume of the average man. These data, therefore, provide a useful indication of the amounts of fluid to be transfused. Thus, for example, with an initial blood volume of 60% of normal, between one and two pint bottles of blood or serum or two pint bottles of plasma should be required to raise blood volume to 70% of normal and restore the blood pressure to 100 mm. Hg or more. When this has been done, a further two or three pint bottles of blood or four pint bottles of plasma should be required to raise the blood volume to 90% of normal.

For the majority of patients who require transfusion, these data provide a satisfactory indication of the amounts to be given. For others, however, particularly those with very large wounds, larger amounts are required, probably because of further bleeding.

f. Time of Operation

Patients with small or moderate wounds, who have not lost much blood, do not need operation urgently but, to prevent the development of infection, it is advisable to operate within six hours of injury. In other cases operation should be undertaken as soon as possible after the circulation is restored. If transfusion is properly carried out, most patients should be well resuscitated within an hour or two of admission. In cases with large or very large wounds it may be necessary to operate even though, because of bleeding or failure of proper response to transfusion, the blood pressure is not restored. Nothing is to be gained and much may be lost by delaying operation on such patients. There is a period of a few hours after injury when the condition of most patients with large and very large wounds can be improved by transfusion, and the best chance of survival is offered by operating within that period, even though the blood pressure is low. The best results are obtained by early operation following early, rapid and adequate transfusion.

These remarks suffice to exemplify the lessons learned during the Second World War. Experience showed that the use of these patterns makes diagnosis more precise and is a better guide to treatment than the use of the prevailing idea of "shock". But much remains to be learned. Thus, though the circulatory patterns are known, the factors provoking them are not fully understood and the mechanisms by which these factors provoke their effects are largely unknown. For example, it is not known how apparently identical sensory and emotional stimuli can produce, on the one hand, the vasovagal pattern and, on the other, the hypertensive, nor how the differing factors of haemorrhage and infection may both give rise to the one pattern of cold hypotension. Knowledge of these mechanisms should advance treatment: for example, it should help in the treatment of cold hypotension due to infection, for which at present no remedy is known, despite the fact that the same pattern when caused by haemorrhage can be cured by transfusion.

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SOME OBSERVATIONS ON GROSS PULMONARY FAT EMBOLISM*

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The experience of the war of 1939–45 showed that most patients suffering from extensive limb injuries and much blood loss could be restored by transfusion. Some, however, died and in the majority of these a gross pulmonary fat embolism was found. Grant & Reeve (1951) attributed death in these cases mainly to haemorrhage, and to delayed and inadequate transfusion. But it remained a possibility that the fat embolism might have been, at least in part, responsible for death, since the occurrence and degree of embolism in those that recovered was unknown. For though, according to some authors, pulmonary fat embolism gives rise to a characteristic train of symptoms, the general view is that its diagnosis is at best uncertain; many cases run their course without symptoms. In the majority of Grant and Reeve's patients in whom gross pulmonary fat embolism was found after death, there was, during life, nothing to differentiate their course from that of patients with only a few or a moderate number of emboli. Since direct experimental observations on the effects of injected fat cannot be made in living man, Armin & Grant (1951) resorted to animal experiment to obtain further evidence. They first sought to define more clearly than hitherto what would be generally accepted as gross pulmonary fat embolism in human lungs, and then determined the dose of injected fat required to reproduce a corresponding degree of embolism in the rabbit, and the effects of this dose on normal animals and on those subjected to a large and measured haemorrhage.

Grading of Pulmonary Fat Embolism

Past writings contain little reference to grading and there is as yet no generally accepted or satisfactory method. Most authors assess the degree of embolism from the histological section and describe it in general terms which indicate the amount of fat. Robb-Smith (1941) recognizes four grades according to the number of fat droplets per unit of area. Armin & Grant (1951) point out that grading is not a simple matter and that for a number of reasons a precise histological definition is not feasible. So far as possible these authors obviate the difficulties by using sections of a standard thickness, by enumerating according to Robb-Smith's method and by illustrating areas sufficiently large to display the general pattern of the embolism. They define gross pulmonary fat embolism in man histologically as that degree of embolism which, in sections cut 15 μ. thick from gelatin-embedded material, falls into Robb-Smith's Grade 4 (several emboli per square millimetre) and gives the appearance shown in their illustrations. To this histological definition they add a definition by extractable fat content, i.e. embolisms having a fat content ranging from 7% to 16%, and on the average about 11.5%, of the dried lung weight. This fat content corresponds to that found by Killian (1931) in human cases in which he holds the fat embolism to have been either alone or in part responsible for death.

Experimental Fat Embolism

Pulmonary fat embolism was produced by injecting rabbit perirenal fat into an ear vein of the unanaesthetized rabbit (Armin & Grant, 1951). It was found that the intravenous injection of only 0.15 ml. (0.145 g.) fat per kilogram of body-weight produces an embolism which, judged both from the stained sections and by extractable fat content, is at least equal to, and on the whole greater than, the gross fat embolism of man.

Effects of Injected Fat on the Animal

This amount of fat (0.15 ml.), injected at the rate of about 0.2 ml. per minute, caused no change in blood pressure, pulse rate or respiration in the normal unanaesthetized animal (Armin & Grant, 1951). This lack of symptoms is in accord with what little evidence there is in the literature. The dose of fat is much less than the fatal dose, which is usually stated to be between 0.5 ml. and 1.0 ml. per kilogram and about half that which Kojo (1922) found necessary to cause a transient dyspnoea. Further, Scriba (1880) found that the degree of fat embolism in the lungs of an animal given a fatal dose was much greater than he ever saw in man, while, according to Cohnheim (1889), filling the lung capillaries with oil to an " incredible extent " causes no alteration in respiration, heart beat or blood pressure.

It might be said, following the work of Ribbert at about the same time (see Ceelen, 1931), that in these experiments healthy animals were used, whereas in injured patients there are added factors. With these factors, a quantity of fat harmless to a healthy man may be deleterious to one not so. Killian (1931) stresses the importance of the combination of fat embolism and shock and blood loss as a cause of death after injury. Armin & Grant (1951), therefore, compared the effects of measured large haemorrhage (averaging about 40% of the blood volume) in one series of rabbits with those in a second series in which the haemorrhage was preceded by the injection of 0.15 ml. fat per kilogram of body-weight to produce gross pulmonary fat embolism. They found that the addition of fat did not alter the clinical picture or increase the mortality (about 20%) due to haemorrhage alone.

The fat-injected rabbit differs from the injured patient in that the factor of tissue damage is lacking. But there is strong evidence that the chief factor responsible for " shock " and death within a day or two of injury is haemorrhage. The haemorrhage to which the rabbits were subjected is of the same order as that found by Grant & Reeve (1951) in patients suffering from extensive limb injuries. These authors conclude that in man the main haemorrhage takes place soon after injury, and it is known that in man pulmonary fat embolism can and often does develop very soon after injury. In Armin and Grant's rabbits the fat was introduced immediately before the haemorrhage, thus rendering the time relation of embolism and haemorrhage similar to that in man.

Finally, since the presence of gross pulmonary fat embolism might indirectly increase the mortality by interfering

Continued at foot of page 18

* Based on the paper by J. Armin & R. T. Grant, published in *Clinical Science*, 1951, 10, 441.

THE PHYSIOLOGICAL AND BIOCHEMICAL DISTURBANCES PRODUCED BY WAR GASES

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1 The vesicants
  a Theories of the mode of action of the mustard compounds
  b Other effects of the mustard compounds
  c Effects of lewisite
  d British anti-lewisite
2 The lung irritants
3 The lethal gases
4 Comment
References

War gases are toxic, though not necessarily lethal, substances whose physical properties enable them to be widely dispersed in the field. Their study is merely a specialized branch of toxicology and they are of interest, not only from the point of view of military medicine, but also because their actions may disturb fundamental physiological and biochemical mechanisms.

These agents are usually classified according to their most obvious clinical effects: e.g., lachrymators, skin irritants or vesicants, lung irritants, lethal gases, etc., but most are capable of producing a varied pathology. It is still convenient, however, to consider them according to this clinical classification in order to see how each group produces its effects.

1. The Vesicants

These include such substances as mustard-gas (di-(2-chloroethyl) sulphide), the nitrogen mustards (e.g., *NN*-di-(2-chloroethyl)-*N*-methylamine; tri-(2-chloroethyl)amine) and lewisite (2-chlorovinyldichloroarsine). All are potent both in vapour and in the liquid phase and they injure every tissue with which they come into contact. They rapidly penetrate the skin to produce erythema, vesication or necrosis, depending on the dose applied.

In the case of mustard-gas and the nitrogen mustards only about 12% of that penetrating the skin remains in the skin; the rest passes rapidly into the circulation (Cullumbine, 1946; Axelrod & Hamilton, 1947). Most of that remaining in the skin is rapidly "fixed", probably by combination with skin protein. The small amount remaining free is detectable only in the epidermis of human skin and even there for only a short time (Cullumbine, 1947). The extent of the vesication produced is proportional to the amount of fixed mustard compound in the skin but, although the fixation occurs rapidly, the vesication may take some hours to develop.

a. Theories of the Mode of Action of the Mustard Compounds

The visible skin damage was found by Dixon, Needham and their colleagues at Cambridge (Dixon & Needham, 1946) to be accompanied by metabolic changes in the skin: for example, a fall in the respiratory quotient and an inhibition of the anaerobic formation of lactic acid from glucose. Other vesicants, as they damage the skin, also produce this inhibition of glycolysis, whereas no inhibition occurs with non-vesicant substances which do not damage the skin. Mustard-gas did not inhibit the glycolysis of hexose diphosphate and this failure suggested that it was the initial phosphorylation of glucose due to hexokinase that was inhibited. This was confirmed and it was also shown that hexokinase was present in normal skin but absent after the development of glycolysis inhibition from skin treated with mustard compounds.

SOME OBSERVATIONS ON GROSS PULMONARY FAT EMBOLISM

Continued from page 17

with the response to transfusion, Armin and Grant, after injecting the same dose of fat, and bleeding to the same degree a third series of rabbits, then restored the withdrawn blood. All the animals quickly recovered and none died subsequently.

The general conclusion suggested by these observations is that, unless the rabbit tolerates fat embolism better than man, in man also gross pulmonary fat embolism is unlikely to provoke symptoms or to be alone or in part responsible for death after injury. In the fat-injected rabbit, the emboli are almost confined to the lungs; in man pulmonary fat embolism is often associated with a considerable degree of systemic embolism. It may be, as Scriba (1880) originally suggested, that illness and death attributed to pulmonary fat embolism may arise from the blocking of systemic vessels, for example, in the myocardium and brain. Moreover, even when such signs as dyspnoea and cyanosis do develop in injured patients, they may be due not to fat embolism but to some other factor. Other factors, such as pneumonia or fractured ribs, are present in many of the published cases; further, it is possible, as Cameron (1948) has recently reminded us, that pulmonary oedema may have a cerebral origin.

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In addition Dixon, Needham and their colleagues showed that all the vesicants they tested, no matter what their chemical nature, could inhibit hexokinase in vitro but that related non-vesicants failed to inhibit hexokinase. Most other enzymes were found to be relatively insensitive to mustard-gas. (With large concentrations many can be inhibited and such a general inhibition may occur in necrosis of the skin, following massive dosage.) It was concluded, therefore, that the integrity of the epidermal-dermal junction is dependent on the continuation of glycolysis through hexokinase activity. When the latter is inhibited, then vesication can occur.

The chemical nature of the reaction between mustard-gas and hexokinase is not known. It is probably just a further example of fixation of mustard compounds by proteins, although hexokinase has no special "fixing" properties when compared with other proteins. Hexokinase is an —SH enzyme, and mustard-gas attacks both the oxidized and the reduced forms.

Hexokinase is inhibited at once in vitro, but in rat skin, for example, inhibition occurs only after a delay corresponding in time to the occurrence of visible skin changes. Therefore the delay in the appearance of vesication still needs explanation.

b. Other Effects of the Mustard Compounds

The mustard compounds do produce other cellular changes. They can cause heritable mutations, can affect chromosome structure and can inhibit mitosis (Auerbach, unpublished work, 1949; Auerbach & Robson, 1946; Auerbach, Robson & Carr, 1947; Fell & Allsopp, 1948a, 1948b, 1949; Slizynski & Slizynski, 1946).

The mitotic inhibition is seen in the pre-mitotic phase of cell division. Higher doses of the mustard compounds will cause scattered nuclear fragmentation and uniform pyknosis, while proliferative cells show alterations in chromosomal structure and function. These latter changes may be reproduced indefinitely in inherited mutations. The mustard compounds react readily with nucleoproteins and nucleic acid; chromosomes are rich in deoxyribonucleoproteins. Similarly viruses containing predominantly deoxyribonucleic acid are very sensitive (more so than hexokinase) to irreversible inactivation by the mustard compounds. This may be a clue to the primary site of action of the mustard compounds, at least in proliferating cells (Herriott, 1948; Phillips, 1950).

During the latent period between contamination and the appearance of macroscopic skin lesions, changes in the tissues are occurring. The permeability of the local skin capillaries is altered within a few minutes, and histological evidence of damage in the dermis and underlying structures can be seen within 20–30 minutes (Cameron & Courtice, 1948). Menkin's capillary permeability factor, "leukotaxine", can be extracted from these damaged tissues (Cullumbine & Rydon, 1946).

The altered capillary permeability results in a loss of fluid and protein from the plasma so that local oedema occurs, the plasma volume and the plasma protein content may fall and haemoconcentration is produced (Cameron & Courtice, 1948). Part of the local accumulation of fluid is drained away by the lymphatic system and this lymph contains increased protein and is also toxic to tissue cultures of bone-marrow fragments. The haemoconcentration lasts about two days in goats and rabbits and then the plasma protein level returns to normal. Then the animal gradually becomes weaker and may die within six to ten days. During this period, diarrhoea and profuse salivation occur in most animals so that there is a loss of fluid low in protein by these routes. This loss results in a second phase of haemoconcentration, this time with no hypoproteinaemia, and the increased water loss also causes dehydration of other tissues, such as the skin and the lungs. The plasma urea concentration rises, oliguria occurs and the urea output increases. These changes involving urea suggest an increased protein breakdown in the body and this must be mainly endogenous, since the food intake is usually decreased and the changes are also seen if food is withheld. The picture is further complicated by the fact that, during the anhydraemia following mustard-gas contamination, the kidneys are less able to excrete the excess urea produced.

Besides these two phases of haemoconcentration, other signs of systemic intoxication occur. The most evident and serious is damage to the haematopoietic tissues (Stewart, 1918; Cameron, Courtice & Jones, 1947; Gilman & Phillips, 1946). There is a cessation of mitosis and a disintegration of the formed elements in the lymphoid tissues, with an accompanying disappearance of the more mature myeloid and nucleated erythroid elements in the bone-marrow and spleen. These tissue changes are reflected by alterations in the cellular content of the blood.

Why the bone-marrow and the intestine (diarrhoea being a symptom) should be the specific sites of action is not known. If mustard-gas tagged with radioactive sulphur is injected intravenously into rabbits, then a little is excreted in the bile and urine but most passes rapidly into the tissues, being fixed there to a maximum within about one to four hours of injection. The greatest amounts are found in the kidney and lung, which show little or no sign of damage, and the bone-marrow contains only about one-twentieth of that seen in the kidney (Boursnell, Cohen, Dixon, Francis, Greville, Needham & Wormall, 1946).

Metabolic changes in the bone-marrow and intestinal tissues can be detected (Dixon & Needham, 1946). There is a marked fall in the anaerobic glycolysis of bone-marrow after a lethal dose of mustard-gas, and this fall parallels the accompanying cellular damage. However, the whole metabolism of the bone-marrow is depressed and there is no specific effect on one particular reaction. A similar decrease in glycolysis is seen in the intestinal mucosa of rats after injection of mustard-gas solutions, but this may be due to the rats ceasing to eat, since an equal change can be found in fasting rats.

The nitrogen mustards are similar to mustard-gas in their general action and their toxic effects (e.g., vesication, inhibition of hexokinase, abnormal capillary permeability, haemoconcentration, diarrhoea, haematopoietic damage, inhibition of mitosis, etc.). Two differences are that (i) in aqueous solution they develop an acute toxicity with a rapid convulsive and lethal action; and (ii) these aqueous solutions remain toxic indefinitely, whereas mustard-gas solutions become non-toxic on standing. The convulsant compounds appear to be ethylenimonium chlorides (Boyland, 1946).

The biological effects of the nitrogen mustards are similar, in many respects, to those produced by röntgen radiation, for example, the intestinal lesions, haematological changes, delayed death, suppression of antibody production, alteration of chromosomes, inhibition of mitosis, and causation of heritable mutations; while certain neoplastic diseases are susceptible to both the mustards and irradiation (Goodman, Wintrobe, Dameshek, Goodman, Gilman & McClennan, 1946; Jacobson, Spurr, Barron, Smith, Lushbaugh & Dick, 1946; Rhoads, 1946; ApThomas & Cullumbine, 1947).

c. Effects of Lewisite

The effects of gross lewisite contamination are also somewhat similar to those occurring after intoxication with the mustard compounds. Thus, locally, vesication occurs in man and skin necrosis in animals. The skin capillaries at the site of application are rendered freely permeable to the plasma proteins so that local oedema occurs, and the lymph draining from the affected area is increased in amount and has a high protein content. A fall in plasma volume is produced and this causes haemoconcentration with hypoproteinaemia. Oliguria, increased urea output and raised plasma urea are seen, which suggests that there is an increased rate of nitrogen metabolism. The period of haemoconcentration is succeeded by one of secondary anaemia, which reaches a maximum about seven to ten days after contamination and lasts for about two weeks (Cameron, Courtice & Short, 1947).

Lewisite can also inhibit hexokinase but there are differences in the gross pathology and the metabolic disturbances produced by lewisite and the mustard compounds. These differences can be attributed to the presence of a tervalent arsenic atom in the lewisite molecule.

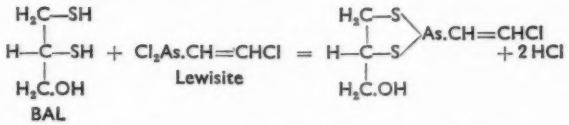
It is not certain in what form lewisite circulates in the body fluids but it is probably fairly rapidly hydrolysed to " lewisite oxide ". A toxic product, probably lewisite oxide, is excreted via the liver and into the bile, producing severe damage to the liver in the process (Cameron, Carleton & Short, 1946). This damage is accentuated by the occurrence of biliary stasis and retention of toxic products, caused by the spasm of the common bile duct that is also produced. Lewisite is also a powerful capillary poison so that, besides the oedema which arises in the contaminated skin, there occur effusions into serous sacs, pulmonary oedema, and haemorrhages in the endocardium, pericardium, alimentary canal wall, serous membranes and in other sites. Many of these pathological changes are similar to those occurring in arsenical poisoning.

Previous work had suggested that the toxicity of arsenicals was due to a selective action on the pyruvate system of enzymes and that an essential SH grouping was the vital point of attack (Peters, 1936; Voegtlin, Dyer & Leonard, 1923). Peters, Stocken and Thompson in the Biochemistry Department, University of Oxford (see Peters, Stocken & Thompson (1945) for review of their work), pursued this idea and soon demonstrated that sodium arsenite and lewisite had a strong inhibitory action on the pyruvate oxidase system[1], that an SH group was essential for the activity of this enzyme system and that lewisite and tervalent arsenicals could combine readily with SH groups in proteins.

This suggested that thiol compounds, by offering readily available SH groups, might compete for the arsenic with the —SH proteins or enzymes. Monothiols proved to be of little value in protecting the pyruvate oxidase system against lewisite, but several dithiols were effective protectors.

d. British Anti-Lewisite

For this purpose the most suitable of these dithiol compounds was 2:3-dimercaptopropanol[2], which has been named BAL (British anti-lewisite) by the Americans. With lewisite or other tervalent arsenicals, BAL forms stable ring compounds, and in this form the arsenic is relatively non-toxic and does not inhibit the pyruvate oxidase enzyme system. The reaction with lewisite is:



Not only can BAL reverse the toxic action of lewisite on the pyruvate oxidase system but it can also prevent it. Further, the in vivo toxic effects of lewisite can be reversed: for example, contaminated animals can be saved by BAL inunction two hours later; vesication of human skin can be prevented by applying BAL up to one hour after contamination; damage to the eye can be avoided by administering BAL up to 20 minutes after contamination. BAL will increase the excretion of arsenic in the urine of the intoxicated animal.

BAL is a colourless liquid and it can be administered by inunction in an ointment base or by intramuscular injection in peanut-oil. It is somewhat irritant itself and has a mild toxicity. Danielli, Danielli, Mitchell, Owen & Shaw (1946) have, however, synthesized the O-glucoside of BAL (called BAL-INTRAV), which can be used for intravenous therapy when rapid detoxification is desired.

This research on vesicants, and especially that relating to lewisite, presents an important fundamental advance. It demonstrates that a biochemical lesion (enzyme inhibition) can cause pathological damage. In the case of lewisite the enzyme blockade can be relieved and the pathological change reversed.

2. The Lung Irritants

As has been noted, the vesicants can also produce large changes in the body-water distribution, because of their effect on capillary permeability. The lung irritants cause similar sudden shifts in fluid distribution (Cameron, 1946, 1948).

The primary disturbance produced by phosgene, for example, appears to be an alteration in lung capillary permeability so that there is loss of fluid with a high content of protein from the circulation and into the pulmonary tissues. This fluid is free of cells and resembles plasma in composition (Cameron & Courtice, 1946). The accumulating pulmonary oedema results in a lowered plasma volume and in haemoconcentration. (The latter can be demonstrated readily in the dog and the goat but may not be seen in rabbits unless they are kept on a dry diet; rabbits seem to have a readily available supply of tissue fluid to meet these emergencies.)

Some effort at draining away this fluid is made by the body; Cameron & Courtice (1946) have shown that, as the pulmonary oedema increases, so the lymph flow from the lung is increased. This drainage route would appear to be the main—if not the only—one available for the resolution of protein transudates from the air passages. Thus Courtice & Phipps (1946) demonstrated that, whereas saline can be rapidly absorbed direct into the lung capillaries from the air spaces, serum was only slowly absorbed and then via the lymphatics. This latter mechanism is slow and cannot keep pace with the plasma outflow in phosgene poisoning. Thus the oedema progresses, the transfer of oxygen is hindered and anoxia is caused.

Phosgene, therefore, like the vesicants, can produce profound shifts in body water merely by acting on the local,

[1] See Peters, *Brit. med. Bull.* 1953, 9, 116.—Ed.
[2] See Peters, *Brit. med. Bull.* 1948, 5, 313.—Ed.

exposed capillary vessels. However, the main toxic action of phosgene is confined to the lung, since it is rapidly hydrolysed in the pulmonary tissues so that none passes into the general circulation. The underlying mechanism of the effect on the lung capillaries is not known. It may be enzymic in nature, especially as Short (see Cameron, 1948) has shown that the mitochondria of the endothelium disintegrate and disappear before the capillaries begin to leak.

3. The Lethal Gases

The inhibition of important enzyme systems is the basis of the mode of action of the so-called lethal gases. The latter can be subdivided further into two groups, viz., (i) those which interfere with tissue oxidative reactions, for example, hydrocyanic acid (HCN), cyanogen chloride (CNCl), and (ii) those which cause auto-intoxication by preventing the destruction of a toxic substance produced by the body itself, for example, di-*iso*propyl fluorophosphonate (DFP) and organo-phosphorus compounds.

Aldridge & Lovatt Evans (1946) have shown that CNCl and HCN have similar physiological effects and that CNCl is converted into cyanide (CN) in vivo. This conversion has been studied further by Aldridge (1951), who has found that CNCl reacts rapidly with both serum and red blood cells but that CN is produced only from the red-cell product. The latter is formed by the combination of CNCl with the globin portion of the haemoglobin molecule, and CN can be liberated from the combination on treatment with glutathione. CNCl itself reacts with glutathione and CN is released if excess glutathione is added. Probably both these reactions (i.e. via the haemoglobin and the glutathione combinations) contribute to the conversion of CNCl to CN in vivo.

The organo-phosphorus compounds produce their effects by inhibiting cholinesterase and so allowing acetylcholine to accumulate peripherally at cholinergic nerve-endings and possibly centrally in the brain and spinal cord. The body is, therefore, allowed to poison itself by its own production of acetylcholine. Death from organo-phosphorus poisoning results from respiratory failure. Respiration is affected in three ways: by spasm of the bronchi, by neuromuscular paralysis of the respiratory muscles, and by paralysis of the respiratory centre. The relative importance of these three effects varies from compound to compound and from species to species, but a direct action on the respiratory centre probably predominates in all cases. The toxicity of the fluorophosphonates was first described by Kilby & Kilby (1947) and the mechanism of respiratory failure caused by organo-phosphorus compounds in general has been recently reviewed (de Candole, Douglas, Lovatt Evans, Holmes, Spencer, Torrance & Nelson, 1953).

4. Comment

No description of the gross pathology produced by the various war gases has been attempted. These have been adequately summarized elsewhere (War Office, 1943). A glimpse of the more fundamental processes involved in the body's reaction to some of the war gases has been given.

Whether gross tissue damage (for example, by the vesicants) or dysfunction (for example, by the lethal agents) is produced, it does appear that some biochemical disturbance may be the fundamental "key" action of the war gases. Even the lachrymators, it has been suggested by Mackworth (see Dixon & Needham, 1946), act by specifically and irreversibly inhibiting enzymes containing essential SH groups.

It is by helping to unravel the relationship between biochemical mechanisms, physiological functions and pathological changes that the war gases have proved to be useful tools to the research worker.

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BLOOD TRANSFUSION AND THE EFFECTS OF INJURY

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1 Traumatic injury
  a Assessment of the severity of haemorrhage
  b Dosage
  c The transfusion medium
  d Rate of administration
  e Practical conclusions
2 Surgical injury
3 Thermal injury
4 " Crush syndrome "
5 Conclusion
References

Blood transfusion shares with reparative surgery pride of place amongst the methods of repairing the effects of injury upon the human body. It is the most satisfactory of all tissue grafts, providing a means of making good the loss of circulating fluid and the cellular and chemical deficiencies in the patient's blood which result from injury. Although a pall of mystery has often shrouded its operation, it is to these simple, but profound, virtues that it owes its value. To understand the precise application of blood transfusion and to obtain from it the greatest benefit demand knowledge of the effects of injury on the physical, cytological and chemical qualities of the patient's blood. It is only when the extent and nature of the defect is appreciated that such fundamental questions as the type of transfusion medium, the quantity required and the rate of administration can be decided.

1. Traumatic Injury

It is impossible to use the term " shock " without becoming lost in a labyrinth of semantics, but there is abundant evidence that the principal phenomena of the general disturbance that follows traumatic injury bear a direct relationship to the severity of haemorrhage and depend upon the consequent reduction in the circulating blood volume (Grant & Reeve, 1951). The effects upon the circulation differ somewhat with the region wounded: in a limb, loss of blood is almost the only factor; in the abdomen there are the additional effects of vomiting and damage to hollow viscera, followed by infection; in the thorax, haemodynamics may be further disturbed by effusion of blood or escape of air into the pleural cavity. The general principles remain the same whether the injury is sustained in civilian life or in warfare, although much of our knowledge has been gained from a study of casualties in battle.

a. Assessment of the Severity of Haemorrhage

The therapeutic role of blood transfusion in the period immediately following a severe traumatic injury is to replace the blood lost. The aim should be to restore the circulating blood volume to a safe level, allowing a margin for further loss during and after reparative surgery. It should be raised to at least 90% of the normal figure. It becomes, therefore, of first importance to have some means of estimating the quantity of blood lost. Measurements of blood volume are clearly impracticable as a matter of routine, but the observations of Grant & Reeve (1951) on wounded soldiers and air raid casualties have provided data of great value.

With injuries of the limbs, provided the main arterial trunk was not severed, Grant and Reeve found that the size of the wound gave the most accurate guide to the amount of blood lost.[1] They used the volume of the hand as a measure: grading wounds as small or less than one hand, medium or one to three hands, large or three to five hands, and very large or more than five hands. With small wounds haemorrhage was usually less than 10% and rarely more than 20% of the total blood volume; with medium wounds the loss was difficult to assess, but usually lay between 20% and 40%; with large wounds it was about 40%; and with very large wounds about 50%. Some guide also could be obtained from the arterial pressure: when the systolic pressure was above 140 mm. Hg, the blood volume was seldom below 80% of normal; when 100–140 mm. Hg, it was above 70%; and when below 100 mm. Hg, it was below 70%.

The other clinical phenomena were inconstant and gave little indication of the quantity of blood lost. Two patterns of disturbance emerged from their observations: the first, with a systolic blood pressure of less than 70 mm. Hg, impalpable pulses, great tachycardia, pallor, restlessness and dyspnoea, indicated reduction of the blood volume to a level at which life could barely be supported—50% or less of normal; the second, with low blood pressure, pallor, cold extremities and bradycardia, was more often attributable to nervous influences than to serious blood loss. In general it is true to say that the earlier the patient is seen after the injury, the more likely are nervous influences to be of importance, and that the pulse frequency tends to be greater in young patients than in old.

With abdominal injuries it is more difficult to assess the quantity of blood lost because the parietal wound gives little indication of the visceral damage. Blood-pressure readings are therefore the only reliable guide. The circulatory disturbance may be further complicated by the effects of vomiting, with loss of water and electrolytes. Infection of the peritoneum is seldom of significance in this respect within the first 12 hours. Thereafter the blood volume may be further reduced by the exudation of fluid rich in protein from the inflamed serosa; this is more likely to happen with rupture of the bowel.

Thoracic injuries again present special problems. The size of the wound gives no indication of the amount of blood lost. Pneumothorax, haemothorax, haemopericardium or a " sucking wound ", i.e., a wound in which air is drawn into the thorax on inspiration, may have mechanical effects upon the circulation which make blood-pressure readings an inaccurate measure of haemorrhage. Haemothorax rapidly excites a serous effusion from the pleural surface, which leads to loss of plasma, and infection of such a collection will provoke further exudation, although neither of these causes is likely to be operative within the first 12 hours.

[1] See Grant, p. 13 of this number of the Bulletin.—Ed.

Short of estimations of the blood volume, laboratory methods are of little assistance in estimating the severity of a haemorrhage. The fallacies of haemoglobin readings are well known, although Grant & Reeve (1951) do not dismiss them as valueless. They found that in healthy young soldiers dilution of the blood began early and that even between 3 and 12 hours after injury the haemoglobin level gave some indication of the deficit. With limb wounds a reading above 90% (14.4 g./100 ml.) meant a loss of less than 40% of the blood volume, and one of less than 75% (12.0 g./100 ml.) a loss of more than 40%. All recent workers agree that haemoconcentration does not occur with limb wounds. In abdominal wounds again a haemoglobin level below 75% (12.0 g./100 ml.) indicated a loss of more than 40% of the blood volume, but with lesser haemorrhages some haemoconcentration might be noted.

The estimation of the quantity of blood lost must be made therefore from an assessment of the size of the wound and the systolic blood pressure, taking into account the complicating effects of abdominal and thoracic injuries. Tachycardia, pallor, a reduced skin temperature, restlessness, thirst and dyspnoea, although frequently present, are of less value in making this estimate. Table I summarizes the points upon which the estimate may be based.

TABLE I. DATA FOR ESTIMATION OF PROBABLE BLOOD LOSS AFTER LIMB WOUNDS

Size of wound	Systolic blood pressure (mm. Hg)	Haemoglobin level (%) (Haldane scale)	Probable loss of total blood volume (%)
Small (less than 1 hand)	140	90	10–20
Medium (1–3 hands) .	100–140	—	20–40
Large (3–5 hands) .	Less than 100	75	40+
Very large (more than 5 hands) . . .	—	—	50+

Estimates of blood loss based on the foregoing considerations cannot be more than a rough guide to the severity of haemorrhage. Moreover, the situation is not stable until all bleeding has been arrested and other causes of loss of blood considered. They do provide, however, an indication of the initial reduction in blood volume and thus offer a means of computing the amount of blood required to restore it to 90% of its normal figure. At this level not only will the acute circulatory disturbances be abolished, but the patient will be fit to undergo reparative surgery. It is probable that further transfusion will be needed during and after operation; judgement as to the necessity for this will depend upon blood-pressure readings taken in conjunction with an assessment of the additional quantity of blood lost during the operation.

b. Dosage

To convert these general principles into terms of dosage requires knowledge of the normal blood volume. It is only within recent years that satisfactory figures have become available. In the past, dye methods have been used to estimate the plasma volume, and the total blood volume has been derived from this and the venous haematocrit value, the assumption being made that the distribution of red blood cells is constant throughout the circulation. Separate estimations of plasma and red-cell volumes have shown this assumption to be incorrect, and when it is made the estimates of total blood volume have been high.

There is good correlation between blood volume and weight and between blood volume and height, and the figures obtained by recent workers have shown close agreement. Those of Reeve & Veall (1949) may be quoted as representative. In healthy adult males they found the mean plasma volume to be 46.6 ml. per kilogram and the red-cell volume to be 30.0 ml. per kilogram, giving a total blood volume of 76.6 ml. per kilogram. The figures for women have been somewhat lower and those for children, with haematocrit values within the normal adult range, about the same as for healthy adult men.

On this basis the normal levels for an average adult man of 11 stone (70 kg.) will be: plasma volume 3,262 ml., red-cell volume 2,100 ml., total blood volume 5,362 ml. In such a man, the probable initial loss of blood from a limb injury would be 500–1,000 ml. with a small wound, 1,000—2,100 ml. with a medium-sized wound, more than 2,100 ml. with a large wound and more than 2,600 ml. with a very large wound. A systolic blood pressure below 100 mm. Hg, or a haemoglobin level below 75% (12.0 g./100 ml.), within the first 12 hours would indicate a loss of more than 2,000 ml.

c. The Transfusion Medium

Many of the systemic disturbances that result from traumatic injury are due to abrupt reduction of circulating blood volume, occasioned by haemorrhage. The transfusion medium is logically, therefore, whole blood. Nevertheless the blood volume can be satisfactorily restored by means of transfusion with plasma, serum, human albumin solution or plasma substitutes such as dextran.[2] The operation is always a matter of urgency and there is no justification for delay in transfusion, which may be started with one of the above while the patient's blood is being tested for compatibility with the blood it is proposed to give. The quantity of whole blood administered must depend in part upon how much is available; although the immediate general effects of injury are due to reduction of blood volume, patients eventually fare better when the haemoglobin level is not permitted to fall too low. Grant & Reeve (1951) consider that it should be maintained above 60% (9.0 g./100 ml.) and Dacie & Homer (1946) 65–70% (9.75–10.5 g./100 ml.). It is reasonable, if blood is not immediately available, to begin transfusion with 500–1,000 ml. of plasma or plasma substitute and change to whole blood when this has been administered.

d. Rate of Administration

In resuscitation after injury the dangers of giving blood too slowly are far greater than any hypothetical risk of giving it too fast. It is impossible to overload the circulation when the blood volume has been suddenly reduced by haemorrhage. The leisurely " drip " method has no place in emergencies of this kind. The first half litre should be given in 5–10 minutes and the second at about the same speed; when the systolic blood pressure is constantly above 100 mm. Hg, the pulse full and the extremities warm, the rate may be moderated. Graham (1944) found that an average rate of 143 ml. per minute was required to resuscitate a patient estimated to have lost more than 2 l.

It is not possible to obtain these rates of administration by a simple gravity method; some positive pressure device will be required. The simplest is a Higginson's syringe attached to the air inlet of the bottle, which will serve to raise the pressure

[2] See Maycock, p. 29 of this number of the Bulletin.—Ed.

of the air above the blood. Some have advocated connecting the air inlet with an oxygen cylinder, a method which makes it possible to deliver 500 ml. of blood in 1½–3 minutes (Pierce, Robbins & Brunschwig, 1949). All positive pressure methods require meticulous supervision, for they carry a greatly increased risk of air embolism. Another convenient device for rapid transfusion is the Henry-Jouvelet rotary pump (Jouvelet, 1934). When even greater speed of administration is required two or three transfusions may be set up simultaneously.

During recent years it has been claimed that intra-arterial transfusion is more effective than intravenous in resuscitating those almost moribund from loss of blood. Russian transfusionists were amongst the first to explore this field, and Radushkievich (1944) claimed to have revived 11 of 13 patients apparently dead or in articulo mortis. Kohlstaedt & Page (1943, 1944) showed that dogs, exsanguinated to the point of death, could be revived by intra-arterial, but not by intravenous, transfusion and that in those bled of lesser amounts only half the volume required for resuscitation by the intravenous route was needed if the injection was made into an artery. Robertson, Trincher & Dennis (1948), working with exsanguinated dogs, found recovery four times more rapid when the blood was returned by intra-arterial, than when by intravenous, transfusion. These observations have been criticized by Case, Sarnoff, Waithe & Sarnoff (1953), who found no evidence that coronary blood flow in the bled dog was increased more by intra-arterial, than by intravenous, transfusion. They concluded that the only indication for intra-arterial transfusion would be a failure of the right ventricle to " pass blood on " to the left.

In spite of its insecure experimental foundation, clinicians are using intra-arterial transfusion with increasing enthusiasm. Its value has recently been reviewed by Seeley & Nelson (1952). Those who have employed it are convinced of its greater efficacy in resuscitation after acute blood loss. Bingham (1952), from experience with more than 100 cases, concludes that intra-arterial transfusion brings about immediate restoration of the coronary, cerebral and renal circulation and raises the blood pressure to normal. Transfusion is usually made into the radial or dorsalis pedis arteries by a positive pressure method with an aneroid manometer incorporated in the apparatus. Blood is driven in at a pressure of 120 mm. Hg with the cannula directed centrally. The cannula is tied into the artery, but, when the radial artery is used, ischaemia of the radial half of the hand has occasionally resulted.

The necessity for cannulation and the dangers of ischaemia have led Haxton (1952, 1953) to use the intra-aortic route. He introduces a long needle into the abdominal aorta from the loin, taking as a surface marking a point on a level with the third lumbar vertebra in the line joining the tip of the left 11th rib and the highest point of the left iliac crest. Blood is delivered at 200 mm. Hg above the mean aortic pressure and 600 ml. may be administered in three minutes. He has used this method with satisfactory results in 20 patients. Intra-aortic transfusion has also been employed with success under direct vision, in patients undergoing abdominal or thoracic operations (Kay & Hacker, 1947; Stephenson & Hinton, 1953).

e. Practical Conclusions

In the resuscitation of a patient who has suffered a severe traumatic injury the immediate task is to restore the blood volume to a level at which the circulatory functions are adequate and reparative surgery safe. This can be done only by transfusion of blood, plasma or some plasma substitute such as dextran. The deficit in blood volume can be roughly estimated by the methods already described, although difficulties occur when the injury is thoracic or abdominal. The aim should be to replace enough fluid to bring the blood volume to between 80% and 90% of its normal level. With simple injuries to a limb, an average 11-stone (70 kg.) man would require not more than 500 ml. if the wound were small, 1,000–1,500 ml. if medium-sized, at least 1,500 ml. if large and more than 2,000 ml. if very large. If the blood pressure is constantly below 100 mm. Hg, he will need at least 1,500 ml. (In the case of small wounds, of course, transfusion will probably be unnecessary.) If an adequate supply of compatible blood is available, it should be used. There must, however, be no delay and, in the absence of compatible blood, transfusion should be started at once with plasma or dextran, of which not more than 1,000 ml. should be given before continuing with whole blood. The transfusion demands an apparatus which allows delivery of 500 ml. in 5 minutes and this rate should be continued until the blood pressure is above 100 mm. Hg, the extremities warm and the pulse full. Transfusion should be continued during operation at whatever speed circumstances may indicate. In patients who appear on the point of death better results may be obtained with intra-arterial or intra-aortic transfusion.

2. Surgical Injury

A surgical operation is a premeditated and calculated injury which, unless due precaution is taken, may be followed by sudden reduction in blood volume and its associated circulatory disturbances. The causes are more complex than in the injuries of warfare or civilian accidents. Nevertheless haemorrhage remains pre-eminent. Careful estimates of the quantities of blood lost at operations have been made and the following figures reveal their, often surprising, magnitude: partial gastrectomy, 267 ml.; abdominoperineal resection of rectum, 411 ml. (Oppenheim, Pack, Abels & Rhoads, 1944); cholecystectomy, 179 ml.; partial thyroidectomy, 668 ml. (Baronofsky, Treloar & Wangensteen, 1946); thoracoplasty, 495–792 ml.; pulmonary lobectomy, 1,607 ml.; pneumonectomy, 1,458 ml. (White & Buxton, 1942); excision of meningioma, 2,000 ml. (White, Whitelaw, Sweet & Hurwitt, 1938). Moreover, in cardiac and vascular surgery, the possibility of unforeseen cataclysmal haemorrhage is always present.

Other causes of loss of fluid complicate the issue: sweating may be profuse under anaesthesia; vomiting may occur; fluid intake may be inadequate; and the toxaemia of acute infection may contribute to the circulatory failure.

Blood transfusion should play a prophylactic part in preventing the circulatory effects of surgical injury. In any operation in which serious blood loss is anticipated, transfusion is started while the operation is in progress and the speed of administration can be regulated to keep pace with the haemorrhage. Intra-arterial or intra-aortic transfusion is of particular value in counteracting the effects of any unexpected and voluminous loss of blood. The importance of water and electrolyte balance is now appreciated by all surgeons.

3. Thermal Injury

A superficial burn entails damage to capillaries and local oedema due to the effusion of plasma from the injured vessels. The volume of this exudate depends upon the area burned and not upon its depth. The initial effusion of fluid is so rapid that the plasma volume may be reduced by as much as 20% within 15 minutes (Lee, Wolff, Saltonstall & Rhoads, 1942). The local fluid loss continues for about 48 hours, reaching a maximum some 36 hours after the injury (Harkins, 1945).

The consequence of this drain of fluid into the damaged tissues is a reduction in blood volume and this reduction is almost exclusively at the expense of the plasma. The resulting haemoconcentration is shown by a rise in the haematocrit value, which may reach 70% (McIver, 1933). Pari passu with these changes in blood volume go the usual circulatory disturbances.

Transfusion has an important place in the treatment of these systemic effects of thermal injury, for it provides a method of restoring blood volume to normal. In this instance the fluid lost is usually mainly plasma, and the logical medium to replace it is often plasma or dextran. The details of treatment are considered in another paper in this symposium.[3]

4. " Crush Syndrome "

Amongst the casualties in air raids during the Second World War were a number of patients who suffered crushing injuries and died in anuric uraemia after a lapse of eight to ten days (Beall, Bywaters, Belsey & Miles, 1941; Mayon-White & Solandt, 1941; Medical Research Council, 1941). The victim of " crush syndrome " had usually been pinned by fallen masonry in such a way that the circulation to one or both legs had been arrested, leading to ischaemic necrosis of a large mass of muscle. When the pressure was released and the circulation restored, an outpouring of plasma into the necrotic muscle resulted and the previously ischaemic limb became tensely oedematous. The sudden loss of plasma led to reduction of the circulating plasma volume, a situation similar to that which follows a burn. These injuries were, of course, often associated with loss of blood from other wounds, but in eight reported examples the haemoglobin readings on the Haldane scale were 160%, 150%, 140%, 124%, 120%, 90% and 92%, on admission to hospital. Haemoconcentration sufficient to raise the haemoglobin level to 150% indicates a deficit of plasma volume of 1,650 ml. in a man of average size. A reduction of blood volume of this order is, of course, accompanied by serious circulatory disturbance.

The value of transfusion in " crush syndrome " is to raise the blood volume to normal levels and to rescue the patient from the immediate perils of circulatory failure. The transfusion medium of choice is plasma or dextran and the quantities required to restore normality can be estimated from the degree of haemoconcentration.

After recovery from the phase of circulatory failure in " crush syndrome ", myohaemoglobinuria usually makes its appearance and is followed by anuria. In most reported instances death from renal failure took place between the 8th and 11th day after injury.

5. Conclusion

An attempt has been made in this paper to give some indication of the value of transfusion in treating the immediate systemic effects of injury in man. The part it can play is vital, but sharply delimited. Its place is to raise the blood volume to levels at which circulatory failure no longer endangers the patient's life and at which the necessary surgical toilet can be safely performed. The deficit in blood volume is usually attributable to haemorrhage, but in thermal burns and crushing injuries the loss is usually predominantly of plasma; in these two instances, therefore, plasma or dextran is often the ideal transfusion medium—in the remainder, whole blood. The best results can be obtained only by careful assessment of the extent of the deficit and the administration of blood or plasma in quantities sufficient to raise the blood volume to 80–90% of its normal level.

[3] See Bull, p. 9 of this number of the Bulletin.—Ed.

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PROVISION OF MATERIALS FOR TRANSFUSION AFTER INJURY

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FOREWORD

SIR ALAN DRURY

At the end of the Second World War, many thought that civilian needs for transfusion therapy would not be considerable; that any problems arising from such needs could be solved by existing knowledge; and that there would be no occasion to think of the resuscitation of large numbers of injured persons. In the event, the civilian needs have increased greatly and there is definite need to consider the resuscitation of large numbers of casualties. This has made imperative the continuance of research.

One of the problems which has always been in the mind of the research worker has been the extension of the useful life of whole blood. Unfortunately, no progress has been or seems likely to be made in prolonging the life of liquid whole blood, but the paper by Mollison, on p. 27 of this number of the Bulletin, indicates that the same result may be achieved by using an entirely different method. If it proved possible to keep red blood cells at −20° C. for long periods of time and if such cells survived well in the recipient, then this method would be of real practical value. By combination of such cells with reconstituted dried plasma, a "whole blood" could be readily prepared from components which were capable of being stored for long periods. In this way the "useful life" of whole blood could be greatly lengthened. Such stores of blood constituents would be especially valuable for major emergencies in areas where blood donors are few in number or transport is likely to be disrupted. It has the added advantage that red cells that are now discarded could be preserved. It might, in time, alter the whole pattern of maintaining stores of whole blood.

Another field of work which has advanced rapidly, and which is dealt with in this number (p. 29) by Maycock, has been the provision of plasma substitutes. There are now available substitutes of proven value. What is more important is that progress has been made in the examination of these substitutes by exact physical and chemical means, and the results are being correlated, as far as possible, with the clinical value of the substitute. It should in time be possible to define much more clearly the "characteristics" required for the ideal blood substitute, and to provide the chemist with data which will lead to the improvement of present-day substitutes or to the suggestion of different substitutes.

The paper on the fractionation of plasma by Mackay, on p. 31 of this issue, is interesting as it comes at a time when the British workers have completed their fractionation procedure. Much work in this field has been done by the Americans, and their results have naturally been of great value to the British workers. The method of ether fractionation, however, differs from the method used by the American workers, and it is interesting to investigators in this field to have two methods which produce fractions having similar biological and physico-chemical properties.

With regard to the risk of post-transfusion jaundice there is much laboratory work in progress on this subject. This work is directed to the sterilization of blood, of plasma, or of blood fractions by physical or chemical means. The use of ultraviolet irradiation, in spite of its initial promise, has proved of doubtful value when used on the large scale and judged by a follow-up survey of patients who had received plasma treated in this way.

The sterilization of plasma and, if possible, of whole blood still remains one of the most important problems for, if it could be achieved, it would remove one of the unfortunate risks of transfusion. It would simplify procedure in the preparation of plasma and plasma fractions, and benefit not only those who receive such material for the treatment of injury but also those who receive γ-globulin for prophylaxis.

RECENT ADVANCES IN THE PRESERVATION OF RED BLOOD CELLS

P. L. MOLLISON

Storage in the Cold at + 4° C.

In the early days of transfusion, blood had to be transferred rapidly from donor to recipient, before clotting had time to occur. The introduction of the use of citrate made it possible to keep blood outside the body, for some hours at least, before using it for transfusion (Hustin, 1914). Within two years, Rous & Turner (1916) had shown that citrate actually had a preservative effect on red cells, for 1% sodium citrate in Locke's solution was better in delaying haemolysis during storage than was Locke's solution alone. However, the effect of citrate was slight and the addition of sugars, particularly dextrose, had a far more striking effect. Rous and Turner showed that not only was haemolysis much delayed in citrate-dextrose solution but also that the red cells retained their viability for at least two weeks. This was demonstrated by producing haemorrhage in rabbits and then using stored red cells for treatment. The transfusion of red cells stored for two weeks prevented the development of anaemia, although red cells stored for three weeks were less effective. Thus, as early as 1916, a method of keeping red cells in good condition for two weeks was known.

However, little use was made of this knowledge until 1940. One reason for this was that the value of blood banks began to be realized only in the late 1930's; another reason was that the method described by Rous and Turner involved mixing blood with relatively large volumes of citrate and dextrose solution so that some supernatant had to be discarded before the mixture could be used. Because of this and because of the fact that when dextrose and trisodium citrate are autoclaved together some caramelization occurs, many blood banks used trisodium citrate alone for blood storage. In 1940 the value of adding dextrose was rediscovered. An important development at this time was the realization that absence of haemolysis in vitro in a suspension of stored red cells did not necessarily imply good survival in vivo (Maizels, unpublished report to the Medical Research Council, 1941; Mollison & Young, 1941, 1942).

Many preservative mixtures were tested, using the criterion of survival in vivo, and it was apparent that viability was better maintained in the original Rous-Turner solution than when a relatively small volume of citrate-dextrose solution was used (Mollison & Young, 1942).

In 1943 various citric acid-citrate-dextrose mixtures were tested in the hope of finding a solution that could be autoclaved without appreciable caramelization. It became apparent that the addition of citric acid to trisodium citrate-dextrose solutions considerably improved red-cell preservation, survival in vivo after a given period of storage being actually a little better than that of red cells stored in the Rous-Turner solution (Loutit, Mollison & Young, 1943). Later it was demonstrated that the good survival of red cells stored in the Rous-Turner solution compared with that of red cells stored with a relatively small volume of citrate-dextrose solution was probably due to the lower pH of blood mixed with the Rous-Turner solution (Loutit, 1945).

Thus by 1943 it had been discovered that the good preservation achieved by the use of the Rous-Turner solution could be achieved more conveniently and could even be slightly improved upon by using a relatively small volume of an acid-citrate-dextrose solution. It must be emphasized that this progress was entirely empirical. Citrate was originally used because of its anticoagulant properties; its preservative effects on red cells are now thought to be due to the fact that it acts as a non-penetrating anion. This effect is important, because at + 4° C. the active " excretion " of sodium by red cells is much diminished and the sodium concentration of the cells increases progressively. Citrate exerts an osmotic force which counterbalances that of the intracellular haemoglobin and phosphate and thus postpones osmotic haemolysis (Maizels, 1943). Dextrose was used originally by Rous and Turner because they thought it would act as a colloid. It is now thought that the good effects of dextrose are due to the fact that it acts as a source of energy and allows normal metabolic processes to continue. However, the extent to which these processes are concerned with the maintenance of the viability of red cells is not known. The pH of the preservative solution was lowered primarily to prevent caramelization of dextrose during autoclaving and not because there was any strong evidence that this would improve viability. It is still not known why adjustment of the pH of blood mixtures to about 7.2 improves preservation.

It remains true that the deterioration of red cells during storage is very poorly understood. For example, it is not known why red cells haemolyse during storage. Since red cells swell during storage and become increasingly liable to lysis by hypotonic solutions, it was at one time thought that haemolysis was due simply to cells bursting. However, this theory had to be abandoned when it was shown that progressive lysis occurred even in solutions which caused red cells to shrink (Mollison & Young, 1942).

It is now generally assumed that the loss of viability of red cells stored at + 4° C. is due to the continuance of metabolic activity. It is known that blood stored at higher temperatures deteriorates far more rapidly; for example, red cells kept at room temperature even for 24 hours have a significantly reduced survival in vivo (Ross, Finch, Peacock & Sammons, 1947). One obvious method of arresting metabolic activity is to lower the storage temperature. However, blood normally freezes at − 3° C., so that, until some method of protecting red cells against the damaging effects of freezing was devised, − 2° C. remained the lowest practicable temperature at which blood could be stored. It is known that this temperature is not low enough, for red cells stored at − 2° C. survive no better than those stored at + 4° C. (Chaplin, Crawford, Cutbush & Mollison, to be published).

Storage in the Frozen State

In 1950 Smith showed that red cells mixed with glycerol could be frozen and thawed without lysis and thus made it possible to explore the effects of low temperatures on the preservation of red cells. Sloviter (1951a) showed that rabbit red cells stored at − 79° C. for six weeks survived well after transfusion. Mollison, Sloviter & Chaplin (1952) showed that human red cells stored for six months at − 79° C. were capable of normal survival after transfusion. In these experiments blood was mixed with glycerol-saline to give a final glycerol concentration of about 15% (w/v). There was progressive lysis during storage so that after six months the recovery of red cells sometimes amounted to only 50% of the number originally frozen. In some further experiments at

−20° C., considerably more rapid lysis was observed; for example, after about three months' storage only 20% of the red cells were recovered. Nevertheless these red cells proved capable of a virtually normal survival after transfusion.

In the course of experiments on the relationship between glycerol concentration and the optimum rate of freezing, Chaplin & Mollison (1953) noted that, when the final glycerol concentration was raised to 30%, rapid freezing was no longer necessary; thus 500-ml. amounts of blood could be frozen without damage by simply plunging the bottles into a bath of solid carbon dioxide and ethanol. Lovelock (1953b) has studied the mechanism by which glycerol protects cells from damage by freezing and has shown that, in the presence of glycerol, salt concentration increases to a lesser extent during freezing. Lovelock (1953a) has shown that there is a critical salt concentration determining cell damage and that, if the glycerol concentration is about 23% (w/v), this salt concentration is not reached (Lovelock, 1953b).

Chaplin & Mollison (1953) noted that, when a citrate-glycerol solution was used instead of saline-glycerol, the rate of lysis during storage was much diminished, and that this was particularly striking at −20° C. It was found that red cells stored with 3% (w/v) trisodium citrate and a final concentration of 30% (w/v) glycerol underwent only about 2% lysis in three months. However, transfusion experiments showed that the red cells, though intact, were not all viable, only about 50% of the number transfused being present in the recipient's circulation after 24 hours (Chaplin, Crawford, Cutbush & Mollison, to be published). Red cells stored at −79° C. for six months survived better, approximately 70% being present 24 hours after transfusion.

Brown & Hardin (1953) carried out experiments on storage of red cells at −70° C. and at −15° C., but they used a higher concentration of glycerol (final concentration about 45% (w/v)) and used sodium lactate instead of citrate. They were able to recover about 90% of the red cells after storage at −70° C. for six months, but, when these red cells were transfused, only about 60% remained in the recipient's circulation at the end of 24 hours. In a single experiment, red cells stored for 50 days at −15° C. had a survival of about 50% at the end of 24 hours.

Thus conditions have not yet been described which allow a good recovery of red cells after long storage, that is to say, a low rate of lysis in vitro, and a good survival in vivo. It might be concluded that this implies that deterioration inevitably occurs and that, even if lysis of cells in vitro is prevented, a proportion of the cells will be found to be non-viable after transfusion. However, work in progress suggests that methods can be found for maintaining the viability of red cells for at least three months, even at −20° C. One way of improving preservation at −20° C. is to leave part of the plasma with the red cells and to use a final sodium citrate concentration of only about 1.5% (w/v) and a glycerol concentration of 30% (w/v). Red cells stored in such a mixture for three months at −20° C. undergo very little lysis and often have about a 90% survival after transfusion. There is evidence that chemical changes are not completely arrested at −20° C. and it is not yet known whether satisfactory storage for more than three months can be achieved (Chaplin, Crawford, Cutbush & Mollison, to be published).

Methods of keeping red cells in the frozen state are not yet sufficiently developed to be useful to transfusion services. However, they have already proved very useful to blood-grouping laboratories, particularly for the storage of cells of rare types. Red cells stored in citrate-glycerol solutions for three months at −20° C. are agglutinated about as well as fresh red cells; after one year's storage the reactions are weaker, but with good antisera the antigens are still easily detectable (Chaplin, Crawford, Cutbush & Mollison, to be published).

Removal of Glycerol from Red Cells

Before red cells stored with glycerol can be transfused, the glycerol must be removed. This was originally accomplished by dialysis (Sloviter, 1951b). Lovelock (1952) showed that glycerol could be more conveniently removed by adding hypertonic citrate and then washing the cells in saline. However, this was not satisfactory when work was being done with mixtures containing as much as 30% (w/v) glycerol, and Chaplin & Mollison (1953) found it better to wash the red cells in several successively more dilute solutions of glycerol. A much more convenient method is to use a continuous washing process such as the one described by Chaplin & Veall (1953), or, better still, the one described by Brown & Hardin (1953). With such a device a bottle of blood can be washed free of glycerol in about two hours.

Conclusion

It is already possible to maintain the viability of red cells for at least six months at −79° C. but it has not so far proved possible to preserve them for more than about three months at −20° C. Red cells stored in the frozen state can be thawed and prepared for transfusion in about three hours. These methods are still at too early a stage of development to be of practical value to transfusion services. However, they hold great promise for the future. Moreover, the new impetus that has been given to research in the field of red-cell preservation may lead at last to a better understanding of the deterioration that occurs when red cells are stored outside the body.

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PLASMA SUBSTITUTES

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A plasma substitute may be defined as a colloid solution which, when injected intravenously, restores a diminished blood volume in the same manner as plasma. Apart from globin and haemoglobin, none of the substances used is derived from human sources.

One of the earliest reports of the use of a plasma substitute is that of Carl Ludwig (1863), who perfused a pig's kidney with a 3% solution of gum-arabic. During the First World War gum-saline and gelatin solutions were given to man for the first time (Hogan, 1915; Hurwitz, 1917; Bayliss, 1916, 1919), and gum-saline continued to be used until reports of reactions and other effects (Studdiford, 1937; Andersch & Gibson, 1934; Dick, Warweg & Andersch, 1935; Keith, Power & Wakefield, 1935) caused its eclipse, perhaps undeservedly.

Interest in plasma substitutes revived in the Second World War and has been sustained mainly for two reasons. (i) The importance of early, rapid, adequate restoration of the blood volume in patients who have suffered injury or haemorrhage has become widely appreciated since 1945. This treatment presents no difficulties where there are transfusion services; but where, for geographical, climatic or financial reasons, there are no transfusion services, blood or plasma cannot easily be obtained. Under these circumstances plasma substitutes, properly used, are invaluable. (ii) There is also the need to consider the means of treating casualties whose numbers might be so great that supplies of the natural transfusion fluids would be insufficient.

A substance that is used as a plasma substitute must possess certain qualities, similar to those of plasma itself.

Positive qualities. When dissolved to make a solution, the substance should exert a colloid osmotic pressure similar to that of the plasma proteins, and the relative viscosity should be similar to that of blood or plasma. Since albumin (mol. wt. 69,000) is the most osmotically active of the plasma proteins, the size, shape and charge of the molecule of the ideal plasma substitute should theoretically resemble those of the molecule of albumin.

The plasma substitute should be retained in the blood stream until it is replaced by the plasma proteins. It should be stable during storage without refrigeration; it should be sterilizable by autoclaving; it should be crystal clear, so that contamination and other changes can be detected; and it should be fluid at temperatures above 0° C.

Negative qualities. The plasma substitute should not be antigenic, or toxic locally or generally. It should not cause permanent histological changes, nor be stored in the body for long periods, and should be eliminated completely. It should not act as a diuretic, and should cause no increase, or only a slight increase, in the erythrocyte sedimentation rate.

The molecular composition is of fundamental importance. Four points have to be considered in the selection of the molecular size:

i. Rate of removal from the blood stream. This is related, at least partly, to the molecular size and shape.
ii. Osmotic efficiency.
iii. Polydispersity of the substance. The degree of polydispersity can be reduced by fractionation, but the fraction selected is always composed of molecules having a range of sizes about a mean.
iv. The association of the larger molecules with certain undesirable effects: for example, rouleaux formation, prolonged storage in the tissues, histological changes in certain organs, and possibly the allergic-like reactions reported in some patients.

The optimum ranges of molecular size of the three most promising plasma substitutes (dextran, gelatin, polyvinylpyrrolidone (PVP)) have not yet been finally determined. Each of these plasma substitutes is made to several specifications and therefore the effects of different solutions of the same substance may differ, and the comparison of clinical and experimental results from different sources is impossible unless the plasma substitute used is fully described.

Agreement is desirable on the optimum specification of each of the plasma substitutes, on the chemical and physical methods used for their measurement and characterization, and on the experimental and clinical methods used for assessing their value as plasma substitutes to restore a depleted blood volume.

Since 1939, many different substances have been examined; of these dextran, animal gelatin, and PVP have been more thoroughly investigated than any others.

Dextran. Dextrans are polymers of glucose, in which more than half of the linkages between the glucose units are of the α-1:6 glucoside type. They are synthesized from sucrose by an enzyme produced by certain strains of *Leuconostoc* spp. Native dextran molecules have molecular weights of the order of millions and are usually branched, the pattern of branching, and thus the molecular shape, depending upon the strain of leuconostoc and the composition of the substrate.

Grönwall & Ingelman (1944, 1945) showed that dextrans given intravenously caused renal damage, though dextran could be given without harm if the molecular weight was sufficiently diminished by hydrolysis; they also showed that solutions of hydrolysed dextran would restore the blood volume of animals that had been bled. Reports of its clinical use in Sweden quickly appeared (Bohmansson, Rosenkvist, Thorsén & Wilander, 1946). Subsequently the results of experimental and clinical investigations in England and in the USA were published (Bull, Ricketts, Squire, Maycock, Spooner, Mollison & Paterson, 1949; Turner, Butler, Smith & Scudder, 1949), and since 1949 a large literature has accumulated.

The clinical solution usually contains 6% (w/v) dextran and 0.9% (w/v) sodium chloride in water. The dextran is prepared by hydrolysing native dextran; the resulting material is a mixture of molecules of various sizes, the range and distribution of sizes being a function of the native dextran used and of the conditions of hydrolysis. Small molecules, small enough to be excreted by the kidneys, and large molecules are then removed by fractional precipitation. The final material is nevertheless always polydisperse and contains a varying proportion of large molecules and small rapidly excreted molecules, depending upon the specification of manufacture. In general, American and Swedish dextrans tend to have a lower mean molecular weight than English dextrans, and therefore leave the blood stream more rapidly.

Dextran possesses most of the desirable qualities of a plasma substitute. After infusion, molecules small enough to enter the glomerular filtrate are excreted in a few hours; the remainder leave the blood at a uniform decremental rate of about one-third per day (Bull *et al.* 1949), and pass into the tissues, especially the reticulo-endothelial system. The dextran

disappears from the tissues at a rate dependent upon its molecular composition, and although traces, detectable only by serological methods, remain for several months, they finally disappear. Minute quantities are also detectable in the urine for several months after chemical methods give negative results (Lorenz & Maycock, unpublished observations, 1953). Dextran thus disappears from the body almost completely in a short time, and although traces may be present in the tissues and urine for long periods, histological changes attributable to dextran have not been observed (Persson, 1952).

The presence of radioactive carbon dioxide in the expired air after the infusion of ^{14}C-labelled dextran in man (Pulaski, 1952) shows that dextran is metabolized, but the metabolic process is not known. An enzyme which reduces dextran to glucose is present in the duodenal mucosa of rats (Bloom & Wilhelmi, 1952) but not apparently in the tissues or blood. The gastrointestinal tract may play an important part in the removal of dextran from the body. After infusion dextran is present in small quantities in gastric juice and bile.

Dextran solutions have two disadvantages. Any solution of which the urinary excretion rate is low (i.e., less than 15% to 20% in 24 hours) is likely to cause rouleaux formation, as long as the concentration of dextran in the plasma is about 1 g./100 ml. or more, and it may be impossible to perform satisfactory compatibility tests. A sample of blood should therefore be collected before the infusion of dextran. Secondly, certain individuals are apparently sensitive to dextran (Maycock, 1952), and may exhibit severe reactions. Such complications are rare and may be explicable by the antigenic relationship known to exist between *Leuconostoc* spp. and certain members of *Pneumococcus* spp. and *Salmonella* spp., or by sensitization to *Leuconostoc* spp. or native dextran. Kabat & Berg (1953) have shown that the native and clinical dextrans used by them act as antigens in man; precipitins and a cutaneous sensitivity of the wheal and erythema type developed three weeks after the subcutaneous injection of 1 mg. of such dextrans in normal individuals. The clinical significance of this observation is not yet clear.

In spite of these disadvantages dextran solutions have been used successfully for the restoration of the blood volume in many countries. The optimum range of molecular size has not yet been defined and it is possible that this will differ according to the strain of leuconostoc used for synthesis.

Gelatin. Solutions of animal gelatin, derived from hides or bones, have been investigated experimentally and clinically in the USA. The native gelatin molecules are degraded by autoclaving, the resultant mean molecular size being dependent upon time of heating. The solution originally tested (mean mol. wt. about 33,000) gelled at room temperature: the next preparation (mean mol. wt. about 19,000) gelled at 12°–15° C. Both these forms of gelatin are rapidly excreted by the kidneys. More recently a polymerized gelatin (oxypolygelatin), with a gelling-point of 10°–13° C., and a " fluid gelatin ", with a gelling-point of 0°–4° C., have been under trial (Pulaski, 1952). All these gelatins are non-antigenic and free of harmful effects—apart from causing rouleaux formation—but their clinical value in the treatment of oligaemia has not yet been established.

Polyvinylpyrrolidone (PVP). PVP is a water-soluble polymer of vinylpyrrolidone, made from acetylene and formaldehyde, which was first used as a plasma substitute in Germany (Hecht & Weese, 1943). For clinical use PVP is prepared as a 3.5% (w/v) solution which exerts a similar colloid osmotic pressure to that of plasma and has a similar relative viscosity. It is stable during storage. Arden, Mandow & Stoneham (1951) have reported a series of 37 orthopaedic patients to whom PVP was given with apparent success, during or after operation, to maintain or restore the blood pressure.

Ravin, Seligman & Fine (1952) have shown that PVP labelled with ^{14}C or ^{131}I is not metabolized to any significant degree in rat, dog or man, that molecules with a molecular weight of less than 25,000 are excreted very rapidly, and that those with molecular weights between 25,000 and 40,000 are all excreted within a few days. They consider that molecules with weights greater than 110,000 are probably retained in the tissues, particularly the reticulo-endothelial system, for a long time—possibly for years. Hüsselmann (1952) has reported histological evidence of storage of PVP and certain regressive changes in human tissues many months after its administration.

The observations of Ravin and his colleagues and of Hüsselmann suggest that any solution of PVP given intravenously should not contain molecules of molecular weight greater than, say, 60,000 to 70,000, the majority having a molecular weight between 25,000 and 40,000. The retention of such a solution in the blood stream would be short-lived. Ravin *et al.* (1952) estimate that in the normovolaemic patient the plasma volume expansion would be only 50% to 60% of the infused volume after six hours, and about 30% to 35% after twelve hours. The place of PVP among the plasma substitutes has still to be determined.

The Use of Plasma Substitutes

Plasma substitutes are not substitutes for whole blood nor are they complete substitutes for plasma, for, among other things, they lack antibodies and clotting factors, have only slight buffering power, and, apart from gelatin solution, do not contain protein and cannot therefore perform the carrier and nutritive functions of the normal plasma proteins. It has been shown, however, that gelatin can act as a carrier and that it can be metabolized.

Thus, in general, plasma substitutes possess only one property of plasma, that of exerting a colloid osmotic pressure. The primary clinical indications for their use are therefore two: the restoration of a reduced blood volume, when the natural fluids, blood or plasma, are not obtainable at all or only after some delay, and the restoration of the osmotic balance when this has been upset as in nephrosis. Their value under the latter circumstances has not yet been established.

When a plasma substitute is used for restoring the blood volume after haemorrhage, the volume given should not be such that the haemoglobin is reduced to below 10.4 g./100 ml. (corresponding to 70% on the Haldane scale). Although restoration of the blood volume is the primary concern in the treatment of haemorrhage, and the repair of the oxygen-carrying capacity of the blood is of less immediate importance, except after massive haemorrhage, patients who have been resuscitated either before, during or after operation by transfusion of blood are probably better able to withstand anaesthesia and resist infection. It is therefore better not to use plasma substitute if whole blood is obtainable.

Bull (1952)[1] has shown that, when dextran is used for restoring plasma volume during the first 36 to 48 hours in

[1] See also Bull, p. 9 of this number of the Bulletin.—Ed.

patients with burns, patients given dextran up to an amount equivalent to their calculated normal plasma volume, followed by plasma—if more fluid is necessary—do as well as patients given plasma. Experience with PVP and gelatin in the treatment of burns is small.

Since all plasma substitutes fail in one way or another to meet all the requirements previously described, and until it is known what importance should be attached to their lack of many of the properties of plasma, they should be used cautiously.

In places where transfusion services are poorly developed or are lacking, in national emergencies, and during the temporary absence of blood or plasma, plasma substitutes have an important part to play.

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PLASMA FRACTIONS IN THE TREATMENT OF INJURY*

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When the part played by concentrated and purified plasma proteins in the treatment of injury is considered, the importance of two groups of proteins is apparent. These are fibrinogen and thrombin, which bring about the coagulation of blood, and prevent protein loss; and serum albumin, which may be used in the treatment of trauma.

Albumin. The colloid osmotic pressure of serum albumin accounts for 80% of that of whole blood, and this property gives it its greatest clinical value. Measurements made by Scatchard, Batchelder & Brown (1944) on material prepared by Cohn (1947) indicated that 1.0 g. of albumin would hold 18 ml. of fluid in circulation by virtue of its colloid osmotic pressure. In acute blood loss Janeway, Gibson, Woodruff, Heyl, Bailey & Newhouser (1944) showed that the injection of albumin brought about an average increase of 17.4 ml. per gram in the circulating blood, although there was a considerable variation in the individual experiments. On the basis of these observations the American workers adopted a standard dose of 25 g. of albumin, and this was thought to be the equivalent of 500 ml. of citrated plasma.

The efficacy of albumin in different forms of injury was shown by Janeway (1944), Cournand, Noble, Breed, Lawson, Baldwin, Pinchot & Richards (1944), and Warren, Stead, Merrill & Brannon (1944). In cases of shock due to severe injury, burns, and surgical operations, the transfusion of albumin brought about a rapid improvement. The injection of albumin in acute loss of blood or fluid caused a transfer of extravascular fluid into the circulation, as indicated by an immediate fall in the haemoglobin concentration and in the haematocrit reading. This was associated with an increased cardiac output. Albumin remained in the circulation for about six hours, when the haemoglobin concentration and the haematocrit value gradually returned to the pre-injection levels.

It was shown that, in traumatic shock, 25 g. doses of albumin may be given at 15–20 min. intervals. Blood should be given at the same time to correct anaemia, and saline to patients who are severely dehydrated. The absence of globulin had apparently no deleterious effects, but it was stressed that the place of albumin in the treatment of haemorrhagic shock was to tide the patient over while blood transfusion was arranged.

Dramatic improvement was noted in patients with burns who were given albumin after the extensive use of saline had resulted in hypoproteinaemia, oedema and the failure of peripheral circulation.

Excessive doses of concentrated albumin are not without their dangers. They may cause pulmonary congestion and oedema, and the limiting factor to the speed at which albumin may be injected will depend on the capacity of the circulation to adjust itself to rapid increase in the plasma volume, and the rate at which the tissues can store or metabolize excess albumin (Janeway, 1944). Martin (1954), in his trial of albumin prepared by the method of Kekwick & Mackay (1954), made careful observations of the overloading syndrome. He found evidence of pulmonary oedema only when albumin was given at or above a rate of 25 g. per hour.

Albumin has a low viscosity compared with plasma, and concentrated solutions are stable in aqueous media. A 25% (w/v) solution of albumin in 0.04 M-acetyl tryptophan may be heated for 10 hours at 60° C., thus destroying the causative agent of serum hepatitis. Concentrated serum albumin formed a large part of the material used by the

* Work undertaken at the Medical Research Council's Blood Products Unit.

transfusion services of the USA during the last war; and with the increased demand for other plasma fractions—particularly if freedom from serum hepatitis could be guaranteed—it will probably play a much larger part in the future British blood transfusion programme.

Fibrinogen and thrombin. Fibrinogen and thrombin, the proteins chiefly concerned with the coagulation of blood, may be purified and concentrated, and these purified proteins, or materials manufactured from them, are used as haemostatics. In the treatment of burns Hawn, Bering, Bailey & Armstrong (1944) claimed that a fibrin film formed in situ by clotting fibrinogen with thrombin prevented oozing from the denuded area, and had no deleterious effects on the healing process. Macfarlane (1943), who used a film made by clotting plasma with bovine thrombin, did not find the treatment practicable, and indeed was of the opinion that the plasma would be better employed as a transfusion fluid.

The fibrin clot may be pressed into sheets and made plastic by heat or by treatment with glycerol. In this form it is used in the repair of dural defects (Bailey & Ingraham, 1944b).

Fibrin foam is made by whipping fibrinogen into a foam which is then converted to fibrin with thrombin, dried from the frozen state, and heated to 130° C. The sponge so formed is a dense matrix of fibrin threads which provide a large surface area on which the effluent blood comes into intimate contact with thrombin, the actual clotting agent. The foam may be left in situ when the wound is closed. Fibrin foam was originally described by Bering (1944), and used first experimentally and then in neurosurgical operations by Bailey and Ingraham (Bailey & Ingraham, 1944a; Ingraham & Bailey, 1944).

During the years 1946 and 1947, foam made by the method of Kekwick and Mackay at the Blood Products Unit of the Medical Research Council, Lister Institute of Preventive Medicine, London, was used by 30 surgeons in 500 neurosurgical operations (McKissock, 1954). It was found most useful in controlling capillary oozing, efficacious in free venous bleeding, and less so in arterial bleeding. A combination of fibrin foam and thrombin was less successful in lung surgery, in which it was used to seal suture lines in the chest walls, to prevent bleeding after the division of adhesions, to cover the bronchial stump after lobectomy and to plug lung cavities.

Mixtures of fibrinogen and thrombin have been used for sealing off small vessels and bronchi in the cut surface of the lung, and to reinforce pleural sutures over bronchial stumps.

In skin grafting, a mixture of fibrinogen and thrombin has been found to be a useful tool. Clotting mixtures are used as an adhesive to hold the skin graft in place on the denuded area. The graft takes satisfactorily, vascularization is observed in three days as compared with a month without the mixture, and there is less pigmentation than with pressure dressings.

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INTERRELATIONSHIP OF METABOLIC CHANGES CONSEQUENT TO INJURY

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The pattern of the metabolic response to injury (e.g., fracture of a long bone, burn, major surgical operation), in particular its effect on protein catabolism, is a phenomenon of basic importance to our understanding of the factors controlling the body's metabolism and is in consequence of fundamental importance to medicine.

1. The Nature of the Metabolic Response

Whilst investigating the mineral metabolism in cases of fracture, the writer discovered a well-marked loss of nitrogen, sulphur and phosphorus (Cuthbertson, 1930). Metabolic studies (Cuthbertson, 1929) on normal subjects splinted in bed had revealed an undoubted but small increase in the urinary output of nitrogen, sulphur and phosphorus, but disuse atrophy could not be considered the main cause of the greatly increased urinary excretion of nitrogen, sulphur and phosphorus that followed injury, nor was it due to the use of anaesthetics. Further observations were then made on fracture cases and on dislocations as soon as possible after admission of the patients to hospital, and on patients subjected to osteotomy, before and after operation.

Patients with moderate to severe physical injury frequently showed oliguria or anuria of varying duration during the first 24 hours; the urinary volume then rose slowly and often irregularly. The earliest specimens exhibited normal amounts of nitrogen, sulphur and phosphorus, but these values soon increased. Occasionally there was a trace of proteinuria. As a rule the maximum excretion of nitrogen was reached between the 4th and 8th day after accidental injury; in a group of post-operative cases (osteotomies, etc.) the peak was reached rather earlier (Cuthbertson, 1932).

The nitrogen : sulphur and nitrogen : phosphorus ratios of the total excess outputs suggested that the substance catabolized corresponded to the ratios found in muscle, but other tissues also present ratios of approximately this order. The maximum daily loss was fairly proportional to the total loss whether estimated over 5 or over 10 days. The total loss during 10 days might even reach as high a value as 137 g. of nitrogen in the case of a fracture of both bones of the leg—with a resulting reduction in the nitrogen content of the body amounting to 7.7%. Such a loss of nitrogen is greater than the nitrogen in the entire liver.

Usually, though not always, the injury produced a rise in body-temperature. The general trend of the temperature curve was parallel to that of the urinary nitrogen but preceded it in time. It rarely exceeded 3.5° F.[1] The pulse-rate curve sometimes lagged behind the temperature but in other experiments there was almost perfect correlation. The basal oxygen consumption usually rose parallel to the increase in nitrogen excretion.

After maximal values had been attained, all the metabolic disturbances declined, but even after six weeks there might be a small but definite excessive loss of nitrogen.

A study of the protein fractions of the blood plasma by precipitation methods revealed a rise in globulins which appeared to be increased by further trauma, and a slight lowering of the albumin fraction. The fibrinogen fraction was often appreciably raised. On the whole there was not much elevation of non-protein nitrogen levels in the plasma (Cuthbertson & Tompsett, 1935).

After fracture of a femur by open operation and without splinting, rats also showed a well-marked disturbance of general metabolism, evidenced by loss in the urine of nitrogen, phosphorus and potassium, and increased creatinuria. Sodium and preformed creatine values remained relatively unaltered or occasionally the level of sodium fell slightly. The wastage of these cellular substances after injury in these rats could not be fully accounted for by the loss of muscle substance from the site of injury or indeed from the injured limb. In addition to the wasting and local autolysis consequent on the injury there thus appears to be a general increase in catabolism, of protein in particular, possibly to meet the enhanced metabolism of the repair process (Cuthbertson, McGirr & Robertson, 1939).

Diets rich in first-class protein, and containing the maximum of calories that the patient could consume, failed to eliminate the negative nitrogen balance at the height of the catabolic process in many cases of fracture due to direct violence. In some cases of injury due to indirect violence where there was not so much tissue damage, nitrogen retention was occasionally observed in patients receiving such surplus diets (Cuthbertson, 1936).

Patients subjected to osteotomy showed less metabolic disturbance than did accident cases: the probable explanation is that the former suffered less pain, because anaesthetics were used and because they were adequately splinted from the start. Further, in these cases the injury to soft tissues was generally less.

A search for accounts of experiences similar to ours revealed a few scattered records in the early literature, which have been described elsewhere (Cuthbertson, 1942), but none of these early workers established that their observations indicated a general metabolic response to injury. This phenomenon of protein catabolism following trauma has now been confirmed by many workers and in other forms of injury, e.g., Lucido (1940), Browne (1943), Cope, Nathanson, Rourke & Wilson (1943), Howard, Parson, Stein, Eisenberg & Reidt (1944), Howard, Winternitz, Parson, Bigham & Eisenberg (1944), Wilkinson, Billing, Nagy & Stewart (1949, 1950a, 1950b) and Moore & Ball (1952). Selye has recognized it as part of what he terms the general adaptation syndrome in stress (Selye, 1940, 1944, 1950, 1953).

Wilkinson *et al.* (1950a) noted in subjects undergoing major surgical operation that the administration of protein

[1] t° F. = $\frac{5}{9}$ (t − 32)° C.—Ed.

(as such), or of protein hydrolysate intravenously, during the period of increased urinary nitrogen excretion, merely increased the urinary nitrogen proportionately and did not modify the " catabolic " phase as described by the present writer in any essential way. This supported the view taken by the writer in his assessment of our knowledge concerning the clinical use of protein hydrolysates and pure amino-acid mixtures (Cuthbertson, 1948).

Howard, Bigham, Eisenberg, Wagner & Bailey (1946) found an excess output of potassium in patients suffering from fractures, the loss being greatest in the first 24 hours, and Blixenkrone-Møller (1949) also found that the greatest amount of potassium was excreted in the first 24 hours after operation. Wilkinson *et al.* (1950b) found that the increase in excretions of potassium and phosphate occurred early in their human postgastrectomy cases and followed a parallel course, as did the increases in excretion of nitrogen and of sulphur which followed later.

Moore & Ball (1952) found that in their cases of injury there was a decreased urinary excretion of sodium for two to five days, followed later by a sodium diuresis. A fall in plasma sodium concentration often accompanied the period of positive sodium balance, particularly where trauma was massive. They considered that the drop in circulating eosinophils, which was of a variable order, and the increase in excretion of steroid hormone products, are evidences of an accompanying endocrine readjustment involving the pituitary and adrenal cortex.

Needham (1952) has recently reviewed other facets of regeneration and wound healing, and for further details of tissue changes this work[2] should be consulted.

2. The Metabolic Response and Vitamin Requirements

The new evidence which supports the theory of an excessive catabolism of body protein also points to an excretion of associated substances, e.g., riboflavin (Pollack & Bookman, 1951).

Browne (1945b) had earlier reported that in burns and in fracture cases there may be almost no urinary excretion of ascorbic acid for up to 21 days after injury, despite a daily intake of both orange juice and up to 700 mg. of ascorbic acid. In a review of the relation of vitamin C to adrenocortical function and stress phenomena, Pirani (1952) found that, in surgical patients, urinary excretion of ascorbic acid markedly decreases during the first few postoperative days. The observations of Kark, Chapman, Consolazio & Nesby (1952) have shown that in man consumption of ascorbic acid by the adrenal gland for synthesis of cortical hormone cannot be considerable and that adrenocortical activity cannot account for the retention of ascorbic acid found after trauma.

3. The Metabolic Response and its Hormonal Interrelationships

The author and his colleagues found that the protein anabolic effect of a crude extract of the anterior pituitary gland could eliminate the excessive nitrogen and creatine loss caused by trauma in rats, though it did not appear to have any effect on the healing process. The effect was general rather than local and might have possibly represented the normal growth response to this extract, superimposed on and thereby masking the increased catabolism following injury, rather than a direct inhibition of the normal response. Certainly no greater rate of restitution of the atrophied muscles of the injured limb was induced; further, the extract had no significant effect on the total time required to heal superficial wounds (Cuthbertson, Shaw & Young, 1941a, 1941b). These experiments demonstrated that the rate of wound healing was not significantly accelerated by a substance which induced an acceleration of general growth—that is, local anabolism was not affected by general anabolism. On the other hand, further observation on rats that were fed on dried thyroid gland indicated that an acceleration of metabolism could induce a more rapid healing (Barclay, Cuthbertson & Isaacs, 1944).

Sellers, You & You (1950) have reported that thyroid function is not apparently essential for the change in protein metabolism after a burn, while the function of adrenal cortex is an important but not an exclusive factor. The effects, on urinary nitrogen excretion, of a mild burn and of exposure to cold were found to be cumulative, and under the conditions of the experiments appeared to be greater than would be expected from the sum of the nitrogen losses due to the two stimuli acting independently (You, You & Sellers, 1950). The total increase in nitrogen loss by rats after burning and exposure to an environmental temperature of 1.5° C. would be made up of a part due to the combined stress, and a part due to the increased metabolic rate that follows exposure to cold. The results of the studies of You, You and Sellers on thyroidectomized and adrenalectomized animals are held to support the view that different mechanisms are involved in the increased nitrogen loss after burning and after exposure to cold. This supports the earlier work on rats of Lathe & Peters (1949), who in addition could not reduce, by methionine supplement, the excess nitrogen excretion due to cold or the excess nitrogen excretion due to burning in the cold.

As against the views of Sellers *et al.* (1950), Gribble & Peters (1951) have found that in rats the excess nitrogen loss following thermal burns is largely abolished by thyroidectomy and that there is a suggestion that the decreased deamination of DL-alanine by liver slices of rats that have been burned is related to the modification of protein metabolism following this injury (van Bekkum & Peters, 1951).

In 1942 Albright first suggested the view that the catabolic impulse of the alarm reaction of Selye may be due to an excessive production by the adrenal cortex of " S-hormone " (now identified as the gluco-corticoids, e.g., cortisone), simultaneously with a decreased production of " N-hormone " (now identified as adrenal testoids). In 1947, Ingle, Ward & Kuizenga found that the negative nitrogen balance that characteristically develops following fractures seems to require the presence of adrenocortical hormones but is not caused specifically by an increase in the secretion of the cortical hormones during stress. Selye (1950) suggests that here we are dealing with a peripheral synergism between the corticoids and other factors produced by stress. Kochakian & Robertson (1951a, 1951b) have shown that in the mouse the metabolic effect of cortisone can be divided into at least two sharply defined phases: a rapid mobilization of readily available protein stores, followed by a period of metabolic readjustment or adaptation. The striking feature of the first phase is the rapid decrease in body-weight and extra nitrogen excretion, and this seems to be concomitant with an involution of the lymphatic tissues. The release of lymphocytes is part of the reaction to injury. While the mobilization of protein from lymphoid tissue is considered by these authors to be a specific

[2] Reviewed on p. 69 of this number of the Bulletin.—ED.

property of the gluco-corticoids, it seems doubtful if the lymphatic tissue is adequate to account for loss of weight and excess nitrogen loss unless there is more lymphatic tissue than we surmise. The eosinopenia has already been mentioned. Ingle (1949) has also reported a protein catabolic phase in rats receiving cortisone, which disappeared after about 10 days. Eliel, Pearson & White (1952) have pointed out that patients in whom hyperadrenocorticism occurred spontaneously, or was induced by administration of adrenocorticotrophic hormone (ACTH) or cortisone, exhibited losses of nitrogen, potassium and phosphorus, strikingly resembling those of operative trauma, a similarity already pointed out by the present writer (Cuthbertson, 1950).[3]

As the period of increased protein catabolism that follows an injury such as a fracture in the rat seems to have much in common with the results of implanting cortisone, it seemed appropriate to determine if the effects of injury and cortisone administration on protein catabolism in the rat were similar in their general character and what would happen if they were superimposed.

The evidence obtained by Campbell, Sharp, Boyne & Cuthbertson (1953) strongly suggests that the effect of fracture of a femur in the rat in terms of increased nitrogen excretion is roughly equivalent to the effect of slowly liberating 25 mg. of cortisone (calculated as the acetate) from the adrenal cortex, and that increasing the stimulus (as measured by cortisone administration) increases the response, possibly on a progressively reducing scale. Whether we can go further and say that the effect of such an injury is to cause the liberation of such a quantity of gluco-corticoid from the adrenal cortex is not so certain. The magnitude of these responses is apparently conditioned by the amount of hormone implanted and by the presence or absence of injury. It seems unlikely that these results with cortisone are exaggerated responses to the injury caused by the operation of implantation in the presence of excess hormone, as might possibly be deduced from the observations of Engel (1952). The similarity in response to two pellets (i.e. 50 mg.) of cortisone to the response to one pellet (i.e. 25 mg.) plus fracture, and the almost identical separate effects of one pellet and fracture, render this very doubtful.

It is difficult to account for these findings in the light of the observations of Ingle *et al.* (1947) to which reference has already been made, unless it is presumed that the effect of injury is not to evoke a liberation of hormone from the adrenal cortex but to cause some change in the circulating hormone which then induces the catabolic phenomenon, and that this effect can be replicated by the changes induced by the implantation of 25 mg. of cortisone acetate.

4. General Considerations

As long ago as 1794, John Hunter wrote with a rare perception: "There is a circumstance attending accidental injury which does not belong to disease—namely, that the injury done has in all cases a tendency to produce both the disposition and the means of cure."

The author (Cuthbertson, 1942) earlier termed the initial period of diminished metabolism immediately consequent on moderate to serious injury the "ebb" period, and the period of increased metabolism of traumatic inflammation, the "flow". The latter is characterized principally by an extensive catabolism of protein which is maximal in most moderate injuries between the 4th and 8th day after injury.

[3] See Addendum, p. 36.

Needham (1952) agrees that this latter period corresponds roughly to the inflammatory defence and demolition period and, therefore, to the anaerobic phase of local metabolism. This phase is accompanied by increased blood sugar, increased body-temperature, pulse and respiration rates, and by other symptoms of fever. It is followed in severe injuries by a temporary anaemia beyond that caused by the actual blood loss itself. Injury is accompanied, as will be described later, by great activity of the adrenal cortex and by the depletion of body protein, which is strangely more extensive than would apparently be warranted by the actual damage and its immediate consequences.

The "ebb" period prior to the "flow" is a period of circulatory depression which is sometimes very manifest in its appearance.[4] Through its circulatory effects this period may have as its first adaptive function the reducing of haemorrhage, pain, and the localization of foreign organisms at the site. But people may die in the course of this reaction and so, provided that means are at hand to combat haemorrhage by treatment with blood or plasma, this should be instituted at once, and adequately.

From teleological considerations the present writer early suggested that the general reaction by which labile protein is catabolized as the result of injury may serve to provide energy or amino acids, or both, for the healing process, and that this is a primitive response independent of food, for a wounded animal is necessarily reduced in its capacity to feed itself. Glycogen and fat depots will also be involved. Certainly carbohydrate consumed after the height of the catabolic period may reduce nitrogen loss (Cuthbertson *et al.* 1939; Lathe & Peters, 1949). Rats on a low-protein diet prior to injury do not exhibit the increase in protein catabolism, presumably because their labile protein reserves are depleted (Munro & Cuthbertson, 1943). On the other hand fasting rats do exhibit it. This difference is probably a matter of time, as the protein-deficient animals were maintained on their diet for a much longer time than rats can be fasted. Munro & Chalmers (1945), continuing this line of work, found that the greater the proportion of protein in the diet of rats before and after injury the greater the amount of nitrogen excreted; the effect of giving a high (25%) protein diet during the period preceding fracture was immediately lost if the animal was put on a non-protein diet after the fracture.

There is no doubt about the ability of fasted animals to heal, and that seems to the writer the most cogent reason for believing that the excessive catabolism of protein, and—one would here also require to presume—of body fat possibly to an even greater extent, is mainly for energy. In the early stages of regeneration the body seems geared to an anti-anabolic phase, and presumably excess food at that stage is wasted in any endeavour to try to reconstitute nitrogen balance. Certainly appetite may be partially lost during this period, particularly if there is a superimposed infection.

Suppression of this "flow phase" of excessive protein breakdown does not necessarily promote regeneration, as we have seen in the experiments with anterior pituitary growth hormone. Thyroid extract, as we have seen, stimulates wound healing. While fasting animals regenerate well, those previously starved for some time prior to injury do not.

Once the phase of repair has begun, added proteins or other components are generally beneficial (Clark, 1919; *Nutrition Reviews*, 1950) and the products of local proteolysis are then

[4] See Grant, p. 13 of this number of the Bulletin.—ED.

utilized for rebuilding (Striganova, 1940). If the supply is restricted, mammals now use it with great economy (Williamson, McCarthy & Fromm, 1951), in contrast to the state of affairs during the "flow" period.

The tissue depletion that follows moderate to severe injury is due essentially to two or more of five main causes: (i) local loss of injured tissues; (ii) loss of blood or exudate; (iii) catabolic response to injury, and sometimes a loss due to (iv) infection; or (v) disuse, or reflex atrophy. There is also a temporary loss of body fluids into the zone of trauma.

Where there has been moderate or serious loss of blood or plasma, nutrition may be held to begin with early, rapid and adequate transfusion. Browne (1945a) and Kremen (1948) have shown that during the catabolic period plasma protein, as such, administered intravenously, does not lead to an immediate rise in nitrogen excretion, and the former goes so far as to say that a patient who is receiving plasma only may be regarded as being starved from this point of view even though he may be in apparent positive nitrogen balance and though the plasma proteins may be exerting their colloidal osmotic pressure. As already mentioned, the writer found that despite substantial increase in the intake of a diet rich in protein and calories by patients with moderate or serious injuries a negative nitrogen balance generally existed at the height of the catabolic period. The nitrogen loss was, however, mitigated by such procedures, a finding comparable to that described by Peters (1944, 1946).

While the patient's metabolism is in a catabolic phase, and the extent of the nitrogen loss is largely a measure of the nutritive state of the organism at the time in respect of protein, there appears to be no special need for concern about this loss in the previously well-nourished subject, provided that steps are taken to replace rapidly and adequately the blood or plasma that has been lost as a result of the accident or operation, and as soon as possible the patient should be gently encouraged to eat a good diet. It is to be remembered that there may be kidney dysfunction during the first day or two and occasionally ulceration of the alimentary tract may result. Peters (1948) has also strongly advised against trying to push nutriment into patients in the catabolic phase and has emphasized that there is no proof that incoming nitrogen can be utilized to prevent the losses consequent on injury at this time.

Every device that can stimulate appetite in the repair phase should be used. This is generally easy if there is no concomitant infection. Where much tissue has been lost there would appear to be a proportionately higher demand for protein, which we know to be best utilized in the presence of abundant carbohydrate: but a lost limb is not replaced as in a salamander. There may also be a special requirement for potassium and for riboflavin and ascorbic acid.

For those about to undergo operation it is strongly advised that within the time available every effort should be made to improve the nutritive state of the body and, after the first few days following trauma, advantage should be taken of the heightened synthetic process in the reconstructive stage so that nothing is lacking for optimal convalescence.

Peters (1946, 1948) has also considered that the negative nitrogen balance following injury and certain other forms of illness is due to an excessive breakdown of existing protein rather than to a failure of anabolism, including failure to utilize ingested protein, though such may also be a factor.

Moore & Ball (1952) are in agreement with the present writer in warning against attempts to cover up the inevitable short-term nitrogen loss by giving unnecessary and poorly utilized proteins and protein hydrolysates intravenously immediately after operation. Whilst pointing out that the net loss of potassium is well tolerated, Moore and Ball, like Wilkinson (1952), are against its being allowed to persist without replacement. On the other hand they advise that alterations in the plasma sodium concentration should not be treated. Like the present writer, they also recommend that a deficiency in intake of energy-yielding food for the first few days following the trauma should not give rise to undue concern: they agree that every effort should be made thereafter to provide a diet of sufficient calories to obtain a positive nitrogen balance at the earliest possible moment. Moore and Ball advise that 25% of the calories in the diet be derived from protein. This is a rather higher proportion than the present writer thinks necessary, but sound evidence for the optimal level of protein is still lacking and it must be noted that some hold that it is sufficient to give a diet with a normal ratio of protein. The National Research Council (1951) advise that all seriously sick and injured soldiers should receive 150 g. of protein daily. Further investigation is required to determine this matter but the answer will not readily be found.

It must be noted that Levenson (1951) believes that if malnutrition can be averted by early high food intakes it seems logical to adopt this precaution and instances the finding that children receiving, from the start, an intake of one-and-a-half times that recommended by the National Research Council as optimal for normals remained in excellent nutritional status following severe burns. Male adults (previously well) receiving, from the start, an intake of 1.5 g. of protein and 45 cal. per kilogram of body-weight show nutritional depletion if the burn is severe—but less than if this intake were not begun very early. Levenson considers that oral intakes begun at these levels on the 1st or 2nd day of injury are well tolerated and that if there is a delay of a few days then gastrointestinal upsets are much commoner. Continuous gastric drip feedings are stated to be better tolerated early than the usual oral feedings. We await further experiences with such dietary measures before advising them generally. They are certainly encouraging.

Addendum

The recent publication of McCann (1953) concerning the hypothalamic control of ACTH release has prompted this addendum. His work suggests that bodily injury results in activation of the hypothalamus either by afferent nervous impulses or perhaps by chemical means, e.g. epinephrine, and that thus a neurohumoral mechanism is released in the median eminence which normally traverses the hypophyseal vessels and causes release of ACTH. He further postulates that in cases of severe stress sufficient amounts of this substance may be released to activate the anterior lobe via the general circulation. This work follows up the observations of De Groot & Harris (1950) on rabbits, and Hume & Wittenstein (1950) on dogs, who have both shown that an intact hypothalamus is essential for ACTH release induced by stress. Porter (1952) has shown that alterations in electrical activity of the hypothalamus are induced by stress stimuli

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EFFECTS OF INJURY ON CARBOHYDRATE METABOLISM AND ENERGY TRANSFORMATION

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1 Shock and carbohydrate breakdown
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In this brief review two problems concerning the role of carbohydrate metabolism in the general reaction to injury are considered. The first arises from studies on the connexion between the injury and the systemic changes that follow, and the second concerns the intimate nature of these changes. The systemic effects of injury will be called " shock ", using that word in the way that the term " inflammation " is used to describe the local changes after injury. If " shock " is used in this way, as a short synonym for " the general reaction of the body to injury ", without an attempt to limit its content or devise diagnostic criteria, the word retains a great sphere of usefulness.

1. Shock and Carbohydrate Breakdown

Although the relation between injury and its general effects has long been studied, little is really known of the biochemical mechanisms concerned. The most that can be done when studying the problem at the present time is to emphasize some important feature of the injury itself, such as haemorrhage. It is, however, particularly remarkable that, no matter how shock is produced, whether by loss of body fluid or by the action of toxic agents, the essential features of the shock state are the same. This suggests that the various initiating mechanisms act through a final common pathway. A possible clue to this came from the work of Engel, Winton & Long (1943), who found that carbohydrate disappeared from the body at an excessive rate in haemorrhagic shock, especially after adrenalectomy. (The terms haemorrhagic, ischaemic and nucleotide as used in this paper refer to the form of the injury.) In view of the effect of adrenalectomy on the resistance to injury and of the action of the adrenocortical hormones in depressing carbohydrate catabolism, Engel (1951) suggested that this excessive destruction of carbohydrate might be the final link in the chain of events connecting the local injury to the general effect. We have recently obtained some evidence supporting this, for we have found that in nucleotide and ischaemic shock in rats the total carbohydrate content of the injured animal disappeared at an excessive rate (Stoner, Threlfall & Green, 1952a; Threlfall & Stoner, 1954). In this context the total carbohydrate content means the yeast-fermentable reducing sugar present either free, as blood and tissue glucose, or derived by acid hydrolysis from glycogen, glucose-1-phosphate, fructose-6-phosphate and fructose-1:6-diphosphate, and expressed as milligrams of glucose per 100 g. of body-weight, the whole animal being used for the determination. Glucose-6-phosphate is not included in the estimation. Separate determinations of this compound revealed an increased level in the undamaged muscle of rats dying from ischaemic shock (Threlfall & Stoner, 1954). It is not known if this accounts for the carbohydrate which is apparently lost. Work is in progress to decide whether the apparent accumulation of glucose-6-phosphate represents an arrest of carbohydrate catabolism or is merely further evidence of the increased amount of carbohydrate being broken down from the stores of carbohydrate in the body. It is clear that injury places a strain on these carbohydrate stores, and the work of Selye & Dosne (1941) suggests that increased breakdown is occurring in the injured area. The resistance of rats to hind-limb ischaemia is increased if their total carbohydrate content at the time of injury is high (Threlfall & Stoner, 1954); this fact is further evidence in favour of the view that excessive destruction of carbohydrate is an important result of injury. An attractive feature of this hypothesis is that it increases our appreciation of the role of the adrenal cortex in the reaction to injury.

2. Shock and Energy Production

All animals, when injured, behave characteristically in that they become very subdued, movement is reduced to a minimum, and the whole body comes to rest, much as in hibernation. The cardinal feature of shock from any injury, in any species, is depression of energy production. For instance, mice kept in a continual state of shock, by the injection of adenosine triphosphate (ATP), show less loss of weight on fasting than do normal controls.

Energy produced by the body appears either as work (metabolic or mechanical) or as heat. In shock an animal remains quite still and a rat or mouse in this condition, if placed on its back, will not even right itself. This absence of mechanical work is not compensated for by any general increase in metabolic work and there is a striking fall in body temperature.

The rate of fall in body temperature naturally depends on the normal metabolic rate of the species and the insulation provided by its skin and subcutaneous tissues. In the mouse and rat the fall in rectal temperature is almost as rapid as after death. In the rabbit it is considerably slower. In clinical practice, where the severity of the injury is usually less than that induced in the laboratory, severe falls in temperature are rare but the depression of the body temperature in man after gross injury has been known for a long time (see Wiggers, 1950). A fall in body temperature means either increased heat loss or decreased heat production. There is no evidence, in experimental shock, of increased heat loss; indeed, the peripheral vasoconstriction and lower respiratory rate that occur in this condition are against such an explanation. In

man, it is thought (Hardy & Ravdin, 1952) that an early decrease in heat loss accounts for the initial slight elevation of temperature which is often seen (Wright & Devine, 1944; Green, Stoner, Whiteley & Eglin, unpublished observations). With Tabor & Rosenthal (1947) we think that the fall in temperature, so strikingly shown by small mammals, is due to decreased heat production, a view supported by the accompanying fall in oxygen consumption and carbon dioxide excretion. Depression of the metabolic rate in shock was first shown by Aub in 1920.

3. Shock and Energy-Yielding Processes

These indications that an essential feature of shock is a fall in energy production focus attention on energy metabolism. Here there seems to be a curious paradox, for, while there is an excessive loss of carbohydrate stores, the animal is not producing even the normal amounts of energy. This could be explained in at least one of three ways:

i. as a widespread destruction of energy-yielding material, the excessive reduction in carbohydrate stores and the final "low energy state" of the animal reflecting an exhaustion of the body's store of energy;
ii. as an "uncoupling" between the production of energy and the oxidation of carbohydrate as, for instance, in dinitro-*o*-cresol poisoning;
iii. as a conservation of the body's energy stores, due to the incomplete metabolism of carbohydrate.

a. Depletion of Energy-Rich Compounds

Potter and his school (McShan, Potter, Goldman, Shipley & Meyer, 1945; LePage, 1946a, 1946b) sought to explain the changes of shock in terms of energy depletion. In a study of the blood and tissues they claimed to have produced evidence in favour of this, namely, a fall in the concentration of energy-rich phosphate ($\sim$P) and of energy-yielding substrates in the tissues, with a rise in the concentration of nucleotide and carbohydrate breakdown products in the blood stream. The most definite evidence was found after generalized tissue damage ("drum shock", described by Noble & Collip (1942), and haemorrhagic shock). When shock followed localized injury (hind-limb ischaemia), energy depletion outside the damaged area was not so evident. In work on the effect of limb ischaemia on muscle phosphates, Bollman & Flock (1944) and Goranson, Hamilton & Haist (1948) found no change in the $\sim$P content of muscle outside the damaged area. This has usually been our experience in shock arising both from this type of injury and from the injection of fatal doses of ATP (Stoner & Threlfall, to be published). Although the phosphocreatine level in the undamaged muscle is somewhat reduced after limb ischaemia, the fall is never gross and at death there are always adequate stocks of readily available energy ($\sim$P) in the undamaged muscle. It might be argued that muscle chemistry is not the best mirror of energy metabolism in this condition. This may be so, but it should be remembered that muscle is the major metabolic tissue of the body (Krebs, 1950) and that it can, on occasion, provide a very good picture of the changes in the distribution of energy (Stoner, Threlfall & Green, 1952b). To overcome such objections these studies are being extended to other tissues but it would not seem possible, by ordinary chemical methods, to exclude the possibility that death in shock might result from the exhaustion of the energy stored in a few vital cells. Certainly, as we have shown above, there is a reduction of energy-yielding substrate (carbohydrate) in shock, but it is clear that death can occur in shock without any widespread depletion of the immediately available sources of energy.

b. "Uncoupling" of Oxidative Phosphorylation

There is a good deal of circumstantial evidence against the second possibility, i.e., that shock is due to "uncoupling". Although "uncoupling" agents may lead to increased glycolysis, they increase oxygen consumption and often raise the body temperature, producing a condition quite unlike shock. More directly, we have shown that after the administration of one of these compounds (dinitro-*o*-cresol) there is a profound fall in the $\sim$P content of the muscle (Stoner *et al.* 1952b). In view of these differences in the physiological effects and chemical results of "uncoupling" and shock, it would seem difficult to explain the changes in shock by this mechanism.

c. Incomplete Carbohydrate Breakdown

This leaves the third possibility, namely, that the animal in shock does not produce energy because, although more stored carbohydrate is being broken down, it is not being completely metabolized. This was the basis of the theory put forward by Long and his co-workers at Yale (Engel, Winton & Long, 1943; Russell, Long & Engel, 1944; Engel, 1945; Wilhelmi, 1948). They were impressed by the disproportionate increase, in haemorrhagic shock, in the blood lactate level in relation to the blood pyruvate concentration, with the consequent progressive increase in the $\frac{\text{lactate}}{\text{pyruvate}}$ ratio. This preponderance of glycolysis over aerobic oxidation implies a swing to the type of metabolism seen in anoxia and, since most of the body's energy is derived from the oxidation of pyruvate in the tricarboxylic acid cycle[1], it is easy to appreciate how such a shift would decrease energy production. As a result the biochemical changes in shock and anoxia have been thought to be closely related. This view of the energy changes in shock arose from the study of a particular form of generalized tissue injury (haemorrhage), and work on shock produced in other ways suggests that these results cannot be widely applied. For instance, although McShan *et al.* (1945) frequently found elevated blood levels of lactate and pyruvate in Noble-Collip "drum shock" and in ischaemic shock, there was no progressive rise in the $\frac{\text{lactate}}{\text{pyruvate}}$ ratio. We have confirmed their findings in ischaemic shock and have also found that a severe degree of nucleotide shock can be present while the lactate and pyruvate levels in the blood are normal (Stoner *et al.* 1952a). The changes described by Long and his colleagues are seen only when hepatic insufficiency is an early result of the injury. The recent fractionation of the blood keto acids by chromatography and ionophoresis (Cavallini, Frontali & Toschi, 1949a, 1949b; El Hawary & Thompson, 1953; Neish, 1953) has shown that the blood pyruvate levels determined colorimetrically, as in all the above work, are too high. Although this will entail a good deal of repetition, the general conclusion, that shock does not represent a simple shift of metabolism towards general anaerobiosis, is unlikely to be altered.

Although the hypothesis of incomplete carbohydrate catabolism cannot be applied in its simplest form, i.e., a block in

[1] See Krebs, *Brit. med. Bull.* 1953, 9, 97.—Ed.

the pathway of sugar breakdown directly between the glycolytic shuttle and the tricarboxylic acid cycle, the hypothesis itself remains attractive in view of the coexistence of excessive carbohydrate destruction with decreased energy production. A more detailed examination of carbohydrate metabolism after injury is in progress, and we now can present only a brief summary of our earlier findings, which are suggestive but not conclusive.

4. Carbohydrate Metabolism after Injury

The changes in carbohydrate metabolism after injury occur in three stages (Stoner *et al.* 1952a). Firstly, the carbohydrate stores are mobilized as a result of the hyperadrenalinaemia which is an early and invariable accompaniment of injury. The effect of this is to convert the cellular stores of carbohydrate into glucose which is transferred to the extracellular space. The hyperadrenalinaemia affects carbohydrate metabolism in at least one other way since it leads to the liberation of adrenocorticotrophic hormone from the anterior lobe of the pituitary. The adrenocortical hormones then released promote gluconeogenesis from protein (Long, Katzin & Fry, 1940), adding still further to the glucose content of the extracellular space. They also apply a brake to carbohydrate catabolism. According to Cori (1950) and his co-workers this might be due to inhibition of the hexokinase reaction that is the first step in the phosphorylation and utilization of glucose.[2] Although the precise point of the block in carbohydrate breakdown in shock is not known, glucose tolerance is reduced after injury, an effect which is dependent upon the presence of the adrenals. In their absence there is no attempt to conserve carbohydrate, and the blood-sugar level falls rapidly after injury. This maintained increase in extracellular glucose constitutes the second phase, which persists until the animal reaches the third stage of rapid death or slow recovery. If death is to be the outcome, a final series of changes occurs just before the end. Probably as a result of the terminal anoxia the blood-glucose level falls and there is a corresponding rise in blood lactate. This change is confined to the vascular compartment and may not have any general significance, as similar changes do not occur in the interstitial fluid.

A teleological view of these findings is that injury creates an excessive demand for carbohydrate in the injured area which, if unopposed by conservation elsewhere, would denude the body of the substrates required for energy production during recovery. Such a conservation is promoted firstly by the secretion of adrenaline, leading to the conversion of the carbohydrate stores to an inert form (glucose) which remains in the extracellular space, and secondly by the secretion of adrenocortical hormones which promote gluconeogenesis and inhibit glucose utilization. This seems to us to be a useful working guide, though anyone who builds hypotheses about shock does so at his peril and must be fully prepared to see them swept away by the advancing tide of biochemical knowledge !

We have found some evidence in favour of this suggested mechanism of adrenal action in our own experiments on the effect of various hormones on the course of shock. Although the great sensitivity of the adrenalectomized animal to injury is well known, irregular results and, for the most part, negative results have been obtained when cortisone and adrenocorticotrophic hormone have been given to normal animals in shock, possibly because the adrenal is already maximally stimulated through the normal channels. However, prophylactic injections of thyroxine in the mouse definitely had a good effect on the course and outcome of shock, this effect possibly being related to the adrenal hypertrophy produced (Green & Stoner, in preparation). Administration of β-naphthoquinone-4-sulphonic acid was also beneficial to the shocked mouse, possibly because of reinforced inhibition of hexokinase. Antagonism of this inhibition with insulin (Cori, 1950) had the reverse effect and greatly decreased the survival time after a standard injury in mice when given in doses of 0.01 i.u. or more per mouse.

5. Shock as a Biochemical Problem

These interesting results give no direct lead to the solution of the central problem, namely, the depression of energy production. The mechanism of this eludes us. It occurs in the absence of the adrenals. Interference with it, as by preventing the fall in body temperature by a high environmental temperature, is harmful (Tabor & Rosenthal, 1947; Green & Stoner, 1950a). Nor does it seem possible to supply energy to the shocked animal in an acceptable form. All of our many attempts on these lines have failed. Although glucose administration will prolong the life of injured adrenalectomized rats, like Rosenthal (1943) we have not found glucose of any value in the normal shocked animal. It might be argued that this is because of the block in its utilization. However, if one circumvents the early stages of carbohydrate breakdown and administers hexose diphosphate or pyruvate, the condition of the animals is made worse. This has been the usual outcome in many experiments with carbohydrate intermediates and metabolic inhibitors of glycolysis (Green, Stoner & Threlfall, in preparation). It seems possible therefore that the depression of energy production represents an additional protective mechanism, perhaps an even more primitive one than that exerted by the adrenal cortex.

We were thus led to look for possible interference at the main site of energy production—the tricarboxylic acid cycle. Little is known of the functioning of this enzyme system in shock. Various in vitro studies, on tissue slices, have been made (Beecher & Craig, 1943; Wilhelmi, Russell, Long & Engel, 1945; Burdette, 1951, 1952) but gross changes have not been found. This is perhaps because the techniques employed were not adequate for the purpose. Since the environmental conditions (for instance, suspending media) in these experiments have been those used for normal tissues, these results may merely indicate the great recuperative power of tissues from animals in shock. In view of the general lack of information on these matters it was interesting to find, in experiments on the whole animal, that the citrate content of the rat kidney was increased in ATP shock (Threlfall, Stoner & Green, 1951). As yet, this is an isolated finding for we have not found a similar change in ischaemic shock. Such results do, however, indicate the possibility of interference with the tricarboxylic acid cycle in shock.

Our work on the general reaction to injury arose out of attempts to explain the mechanism of nucleotide shock which, in turn, arose from the finding that ATP is the active principle of Dyckerhoff's " myotoxin ". These findings were made in the course of a search for possible metabolic factors in the causation of shock, carried out in the early part of the Second

[2] See Dixon & Webb, *Brit. med. Bull.* 1953, 9, 110.—ED.

World War. They posed two problems: the relationship between the state produced by the injection of adenine nucleotides and naturally occurring shock, and the possible role of these compounds in the production of the natural condition. Both problems are still mainly unanswered. While it seems justifiable to describe the condition produced by the injection of nucleotides as a form of shock, bearing in mind the meaning we have given to that term, their role as shock-inducing metabolites is still very problematical. From our current work we are not able to add anything in this context to our previous writings (Green & Stoner, 1950a, 1950b). It should not be concluded, however, that the discovery of the mechanism of nucleotide shock would not prove to have some bearing on the main problem.

In this short review a number of problems have been introduced. None of them has been fully answered nor is it likely they can be in the present state of knowledge. Many more experiments are necessary before any of the hypotheses that have been put forward can be accepted as proven but so far as they go they support the general thesis that shock is primarily a state of diminished energy output; and if this is ever firmly established the approach to the whole problem, both theoretically and practically, will be radically altered in many ways.

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REACTION OF ENZYMES TO INJURY

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The subject under review—the reactions of tissues to injury, as reflected in the reactions of their enzymic systems—is vast. A great amount of work has been done in studying the effects of various poisons on the enzymes of the cell, and these experiments have not only shown us how the poisons act, but have also yielded much information about the enzymes themselves. It is not proposed, however, to consider here such things as the effect of cyanide on the respiratory activity of cells, the effects of heavy metals on enzymic systems, and so on. The action of drugs on enzymes has been reviewed by Quastel in a recent number of the Bulletin[1], in which Sir Rudolph Peters[2] has also described the work of his group on arsenical poisoning and on the action of fluoroacetate. These studies are classical; in a review of this type we must be concerned with examples which illustrate principles, and there is little point in considering here further examples of similar work. Also for these reasons, the work of Dickens (1946), on poisoning of respiratory enzymic systems by high oxygen pressures, has not been included in this review.

It is intended in this paper to take a rather wide view of injury. Included in the topics for discussion are mutations and micro-organisms; metabolic disorders, inborn and acquired; radiation injury; Chastek paralysis (an example of an induced deficiency state); the effects of two poisons, i.e., 2:4-dinitrophenol and carbon tetrachloride, whose mode of action does not conform to any previously known pattern; and inflammation and repair. Although most of the work to be discussed was performed in vitro, some in vivo studies, particularly those relating to biosynthetic processes, are included.

* This review was prepared during the tenure of a Beit Memorial Fellowship.
[1] See Quastel, *Brit. med. Bull.* 1953, 9, 142.—Ed.
[2] See Peters, *Brit. med. Bull.* 1953, 9, 116.—Ed.

The present writers feel that ultimately the study of disease may evolve itself into the study of the dynamic biochemistry of the cell. Viewed from this point, it may be said that the investigation of enzymic reactions to injury has scarcely begun.

1. Genetic Injury and Enzymes

a. Microbial Mutations

The important work of Beadle and Tatum (Beadle, 1945; Beadle & Tatum, 1941; see reviews by Pontecorvo, 1950, Horowitz & Mitchell, 1951, and Beadle, 1948) led these authors to raise again the subject of the relationship between genes and enzymes, and to postulate a one-to-one qualitative relationship between them, i.e., one gene representing or controlling one enzyme. This work with *Neurospora* has shown that a bewildering series of mutants, differing in their nutritional requirements, can be obtained. Nevertheless, the exact correlation between these differences and the presence or absence of a particular enzyme has been demonstrated in only a few cases. It is worth considering a particularly elegant example of this type of correlation. Mitchell, Houlahan & Lein (1948) showed that a *Neurospora* mutant could be obtained which grew on tryptophan, but not on indole. Previous work by Tatum & Bonner (1944) and by Umbreit, Wood & Gunsalus (1946) had shown that *Neurospora* strains could synthesize tryptophan from serine and indole, and that pyridoxal phosphate was a coenzyme of this synthesis. Mitchell *et al.* (1948) made cell-free extracts of their mutant and showed that the enzyme catalysing the condensation of serine and indole was absent from them, while present in non-mutant strains. In other words, the mechanism of the genetic failure of this reaction was due to absence of the enzyme.

This is indeed a subtle process of cell-injury, and it is unfortunate that the validity of the correlation can be questioned. In short, the objection to such experiments can be put as follows. Is a technical difficulty being mistaken for a general principle? Is it possible that the enzyme is in fact present, but that for some reason it is not possible to demonstrate its presence in the cell-free extracts? Many examples of such difficulty are known and have led to errors of interpretation in the past. It is quite conceivable that the enzyme may be present within the cells, but may be useless to them either because some other substance required by the reaction is absent or because an inhibitor of the reaction is present.

For example, Wagner (1949) has found that a strain of *Neurospora* that requires pantothenic acid is unable to carry out the condensation of β-alanine with pantoyl lactone, both moieties of the pantothenic acid molecule. Nevertheless, he has found that the enzyme catalysing this reaction is present within cells of this mutant strain.

These beautiful studies with micro-organisms are fully illustrative of the theme of this paper. They show the tremendous possibilities of attacking problems of abnormal physiology of cells by methods of enzyme chemistry, and they illustrate the gaps and pitfalls of the methods. Nevertheless, it is clear that the advancing analysis will in the end demonstrate the nature of the defects caused by the mutations.

b. Inborn Errors of Metabolism

Another good example of a similar investigation is to be found in the work of Gibson (1948), who studied the familial human disorder known as methaemoglobinaemia. In this

rare disease abnormally large amounts of methaemoglobin accumulate in the blood and it is known that the addition of methylene blue either in vitro or in vivo results in the rapid removal of the abnormal pigment. Gibson studied the reduction of methaemoglobin and found that this reaction is coupled to the action of dehydrogenases that require coenzyme I (diphosphopyridine nucleotide, a molecule which contains nicotinamide and adenylic acid). An essential in the reduction of the pigment is the presence of " coenzyme factor I ", this being responsible for the transfer of hydrogen from reduced coenzyme I to methaemoglobin, and it further appeared that the blood of sufferers from familial methaemoglobinaemia was deficient in this factor.

Thus this study suggests a satisfactory mechanism for the disease in terms of enzymic reactions. It is subject to the objections that we have already applied to the experiments with *Neurospora*, but again the way to further analysis has been opened. The original paper (Gibson, 1948) should be consulted so that the logic and elegant application of method can be appreciated.

c. Radiation Injury

A final example of the interrelationship between genetic change and biochemistry can now be considered. This concerns work on the effects of ionizing radiation on chromosomes and some parallel work on radiation injury of enzymes. It is by no means as satisfactory as our previous examples, but indicates the future possibilities.

Thoday & Read (1949a, 1949b) showed that root tips of *Vicia faba* exposed to ionizing radiations suffered more chromosome damage in oxygen than in nitrogen. Giles & Riley (1949, 1950) found the same sort of effect in Tradescantia microspores, there being a fivefold increase in damage in oxygen as compared to nitrogen. Barron and his co-workers, in a study of the action of ionizing radiations on enzymes (Barron & Dickman, 1949; Barron, Dickman, Muntz & Singer, 1949), found that those enzymes possessing SH groups as their active centre were sensitive to x rays in dosages below 5,000 r. Irreversible inactivation was often produced. Non-SH enzymes were far less sensitive. It is of interest that absence of oxygen afforded some protection against this injury and that glutathione was also effective in this respect. Evidently such radiations produce their effect by the oxidation of sensitive groups such as SH, and these observations hold out obvious hopes for treatment.

2. Deficiency States

The now classical work of Peters (see footnote in the introduction to this article) showed the nature of the defect in thiamine deficiency, and he put forward the idea of the " biochemical lesion ". The passage of time has served to emphasize the importance of these ideas, and we now examine a curious example of a deficiency state in animals, the investigation of which is a model of how field work on a sporadic disease led on to biochemical study that demonstrated beyond doubt the nature of the lesion.

a. Chastek Paralysis

In 1934, a paralysing disease of foxes was encountered on the Minnesota farm of a Mr J. A. Chastek. The clinical features of the disease included a refusal to feed, followed shortly by a weakness of the muscles, progressive paralysis and death. Green (1936) reported on this condition, and named it Chastek paralysis. After much investigation, it became clear that the animals were suffering from thiamine deficiency (Green & Evans, 1940). The nature of this deficiency is an extraordinary one. It was found that the outbreaks occurred at a time when the foxes were being fed raw fish as a source of protein, and examination of the fish showed that it contained a factor which inactivated thiamine. This substance was heat-labile, and turned out to be an enzyme, thiaminase. Woolley (1941) made from the tissues of the carp highly active preparations of thiaminase, which destroyed thiamine in vitro, and a little later Krampitz & Woolley (1944) isolated the split products of the thiamine molecule after enzyme attack and demonstrated beyond doubt the enzymic nature of the Chastek paralysis factor. Thus the ingestion of a destructive enzyme caused such depletion of thiamine that the animals were rendered deficient in the vitamin.

The final state—in this instance, paralysis—was but an indication of the site of enzymic failure, and only after this failure had developed did the gross pathological changes appear. In this instance, first the pathology was determined, then the site of injury was defined and finally the operating cause was established. The possibility that many diseases are of this type is a very attractive one—one that holds out hope for the rational treatment of lesions that we now recognize only by the destruction and the irreversible changes which they bring about, with no clue as to the underlying mechanism.

3. Action of Toxic Substances

a. 2 : 4-Dinitrophenol

Two substances will be considered in this section. The first of them is 2 : 4-dinitrophenol (DNP); this compound is one of a family which includes dinitro-*o*-cresol (DNOC) and which has excited a good deal of interest in recent years. These substances have powerful pharmacological effects. When given to animals (including man) they produce a great increase in the basal metabolic rate; the heat production of the body rises so sharply that hyperthermia is induced and, if the poison is administered over a long period, the great metabolic activity is accompanied by a greatly increased appetite and by rapid wasting.

DNOC had, for this reason, some vogue as a weight reducer in clinical practice. Its use was discontinued because its toxicity was great and because acute poisoning appeared without warning and quite irregularly. In recent years, DNOC has been used against the locust, and in Britain and elsewhere as an insecticide and herbicide. Its importance is twofold. In the first place, its toxicity to man and its widespread use make it necessary to know what one can of its mode of action, cases of poisoning being not uncommon; in the second place, its effect on the body is obviously a rather subtle one, and presents a challenging problem.

It has been known for some time that DNP and DNOC, when incubated with cells such as the sea-urchin's egg, cause a vast increase in their oxygen consumption. This picture of great metabolic activity was accompanied, however, by a virtual cessation of growth, the cells failing to divide in the presence of the poison. Chemical studies of organisms such as yeasts showed a similar picture. Oxygen consumption was increased, but the excessive combustion of foodstuff was associated always with a failure of useful activity. In the case

of yeast, this was reflected by a depletion of reserve materials such as starch, and a failure to synthesize fresh reservoirs. Evidently, DNOC and DNP effect a separation, to put it broadly, of anabolism and catabolism. Breakdown proceeds unchecked at an accelerated pace; building-up is at a standstill.

The enzymic nature of this failure has become clearer in the last few years. It would be as well to explain briefly the present ideas on the methods by which cells use the energy which they generate by the breakdown of the carbohydrate, fat and protein that reach them. It is obvious that on combustion of a molecule of glucose (for example) a considerable amount of energy is given up. In the cell, this combustion takes place step-wise and in a controlled fashion. The mechanism of this breakdown is now fairly well established. During the breakdown which takes place in a series of oxidations and splittings of the glucose or other carbohydrate molecule, it has been observed that inorganic phosphate is taken up from the medium and converted into compounds such as adenosine triphosphate (ATP) and creatine phosphate. It has also been observed that these compounds require considerable energy to be formed, and that, once formed, they will readily break down again and liberate the energy which has been put into them. In other words, during biological oxidations, the energy liberated from the molecules burned is not dissipated as heat, but is stored in the form of special compounds which can be used later in much the same way as an electrical battery may be charged at one time, and the energy so received made available at another convenient time.

This process of phosphorylation associated with oxidation can very easily be studied in vitro. Cell-free preparations are available that will undertake the complete series of oxidations known as the Krebs cycle or tricarboxylic acid cycle and that at the same time will use the energy made available to synthesize ATP.[3] It has been shown (Loomis & Lipmann, 1948; Cross, Taggart, Covo & Green, 1949; Judah & Williams-Ashman, 1951; Judah, 1951) that DNP and DNOC will act on such preparations in a way that might be expected from the observations on whole cells. They permit the oxidations to continue, but they prevent the use of the energy so liberated, with the consequence that no ATP is synthesized in their presence. Since ATP is required as a source of energy for numerous synthetic reactions (which can also be demonstrated in vitro) such as protein synthesis, phospholipid synthesis, etc. (see, e.g., Williams-Ashman & Kennedy, 1952), these synthetic reactions are also brought to a standstill, as has been shown experimentally. In other words the dissociation of the breakdown process from the building-up process in the presence of nitrophenols has been traced to the inhibition of the phosphorylation reactions.

Judah and Magee, in unpublished experiments, studied the effect of DNP on the contracting diaphragm of the rat in vitro. They observed that, after treatment with DNP, on electrical stimulation of the nerve-muscle preparation or on direct stimulation of the muscle, contractions were elicited normally for a short time, after which their amplitude diminished and finally contractions virtually ceased. At the same time, a state of rigor developed and reached its maximum at the moment when contractions ceased. Chemical estimations showed that, during this time, both the creatine phosphate and the ATP had reached vanishing-point.

[3] See Krebs, *Brit. med. Bull.* 1953, 9, 97.—ED.

The dissipation of these compounds also occurred if the muscle was allowed to remain unstimulated in the presence of the nitrophenol. The muscle soon became unable to respond to stimulation and went into rigor. These changes were, for the greater part, irreversible once the state of rigor set in. Washing out of the DNP resulted in a partial return of activity.

The observations recorded here show in a reasonable way how the nitrophenols act. It has not been possible at the time of writing, however, to state exactly the enzymic mechanism of this action. That is, we are not able to say at what point in the complex phosphorylation system they do their work. The nitrophenols have proved valuable tools in the analysis of this system, and the solution to their problem and the exact definition of the phosphorylation mechanism are interdependent problems.

b. Carbon Tetrachloride

Carbon tetrachloride has long been known to produce liver injury (see Cameron & Karunaratne, 1936), but its mechanism of action has been obscure. Carbon tetrachloride is of interest not only because of its tissue specificity for liver, but because it is a rather inert molecule, and it seemed difficult to visualize its action in terms of enzymes. Christie & Judah (1954) have attempted to do this very recently, and have put forward a hypothesis which seems to provide a reasonable mechanism of action.

Since 1948, a method for the isolation of cell components such as nuclei, mitochondria and submicroscopic particles (microsomes) has been in use in many laboratories (see Schneider & Hogeboom, 1951, for a review). In the experiments on carbon tetrachloride Christie and Judah used these methods extensively to find that CCl_4 administered to rats apparently attacks the mitochondria of the liver directly. These particles are known to carry the enzymes responsible for the oxidation of the substrates of the tricarboxylic acid cycle and also for the phosphorylations (energy-transforming systems) associated with these oxidations (see Schneider & Hogeboom, 1951; Christie & Judah, 1953).

It seems that the attack on the mitochondria by CCl_4 results in considerable injury to the enzyme systems, and it is important that CCl_4 can be shown to have effects in vitro on isolated mitochondria similar to those after its administration to the whole animal.

Choline, which is known to ameliorate the acute effects of CCl_4 on the liver, is effective in reversing partially the action of CCl_4 on the various mitochondrial enzymes studied; other experiments performed by Christie & Judah (1954) also helped to correlate biochemistry and pathology and it seems that the proposed mechanism of action of CCl_4, i.e., a direct attack on the liver mitochondria, is at least reasonable, if not proved conclusively.

It appears that a poison is acting in this case not by attack on a single enzyme, but by attacking the physical structure on which the enzymes are carried. The specificity of the site of injury is worth considering. The liver is specially involved but it is a fact that agents such as CCl_4 will destroy the mitochondria of any tissue if allowed access to them in vitro. It would appear that factors other than a simple chemical affinity are important here. For example, the method of transport of CCl_4 in the blood stream may be significant in determining its site of action.

4. Inflammation and Repair

Work by Menkin (1940), Duthie & Chain (1939), Cullumbine & Rydon (1946) and Spector (1951) has shown that certain peptides have the property of producing some of the effects of acute inflammation in vivo. These effects are increased capillary permeability, swelling of vascular endothelium, and emigration of leucocytes. The significance of the observations is that destruction of tissue is a feature of different types of inflammation that, despite the similarity of the tissue changes observed in them, otherwise have nothing in common. Clearly such a destruction of tissue might lead to breakdown of protein with formation of peptides; indeed evidence for the process exists in the work of the authors cited above.

It seemed likely that production of peptides in injured tissue was most probably due to the action of proteolytic enzymes. It is now known that such enzymes exist in tissues, for example, in skin (Beloff & Peters, 1945). A proteolytic enzyme similar to trypsin is also present in human plasma (Christensen & MacLeod, 1945) and has been studied fairly extensively. The enzyme is normally present as an inactive precursor but can be activated by chloroform, by certain ions, for example, Ca^{2+}, and by kinases, present in platelets, streptococci, cells and serum. The activity of the plasma proteinase, or plasmin or fibrinolysin as it is also called, may be controlled by a system of inhibitors—antiplasmin, which is removed by treatment with agar (Roche e Silva, 1952)—and activators. A system of this kind would presumably resemble the mechanism which controls blood clotting.

It has been suggested (see review by Gell, 1953) that activation of plasmin may occur in injured tissues as a result of formation of transient platelet thrombi and associated phenomena. The reason for this is that such aggregates are known to be associated with the release of histamine, and it now seems likely that peptides may cause increased capillary permeability by liberating histamine (see Miles & Miles, 1952). The peptides themselves, in this hypothesis, would be formed by the action of activated plasmin on neighbouring protein, for example, fibrin. Another possible effect of activation of tissue or plasma proteinases by local injury is the splitting of protein mucopolysaccharide complexes in ground substance, with consequent liberation of free hyaluronate. The hyaluronate might then be more easily depolymerized and hydrolysed by tissue hyaluronidase, with the resultant well-known spreading effect. It is also possible that injury may itself liberate or activate tissue hyaluronidase (see Meyer & Rapport, 1952).

After the destruction seen in acute inflammation comes repair, with formation of new ground substance, collagen, and of more specialized tissues. Layton (1950) compared the rate of fixation of inorganic isotopic sulphur in normal and granulating tissues. He found a great increase in the amount of sulphur bound by the latter tissue compared with that bound by the former. The figures for granulating tissue were in fact comparable with those the author obtained for embryonic tissue. Layton interpreted his results as evidence for greatly accelerated biosynthesis of sulphur-containing ground substance during the repair stage of inflammation, and found the acceleration to be inhibited by cortisone, which is known to delay healing in vivo (Layton, 1951).

5. Injury and Protein Biosynthesis

To study the biosynthesis of a protein in vivo with the now universally employed isotope technique, it is often necessary to isolate the protein in a relatively uncontaminated state. For this and other reasons, much of the work in this field has concerned blood and plasma protein.

a. Acute Inflammation

Yuile, Lucas, Jones, Chapin & Whipple (1953) investigated the turnover of plasma albumin and fibrinogen, and of the protein of red blood cells, in dogs with experimentally induced sterile inflammatory abscesses. They found a great increase in the rate of synthesis of fibrinogen and a less striking increase in the turnover rate of plasma albumin. They also observed a marked decrease in the rate of biosynthesis of the protein of the red cells, presumably mainly haemoglobin, and suggested that this latter finding may be connected with the anaemia that often develops during the course of inflammatory conditions.

b. Starvation

An interesting observation on protein synthesis has been made by Miller, Bly, Watson & Bale (1951). Using semi-starved dogs, they found that when plasma labelled with radioactive carbon was given parenterally, the animal, at the end of a week, still retained 90% of the radioactivity. When, on the other hand, the labelled plasma was given by mouth, two-thirds of the radioactivity was excreted within 24 to 48 hours. These experiments suggest that, in dogs in a state of semi-starvation, if plasma protein molecules can be introduced intact into the circulation, the protein will enter cells without being previously degraded, and that the constituents of the plasma protein molecules will be used over and over again without being excreted. Thus it may be that a condition of negative nitrogen balance is associated with changes in the enzyme systems concerned with protein metabolism.

6. Disturbances of Plasma Protein Metabolism

When an amino acid labelled with an isotope is given, and the rapid incorporation of the isotope into the plasma proteins that follows is plotted against time, a characteristic curve is obtained. To try to interpret these curves, it is necessary to study their ascending limb, the peak value of isotope content, and their declining limb. Abnormally shaped curves may be due not only to changed rates of synthesis and degradation, but also to alterations in the total amount of the protein that is in the body or is available for labelling.[4]

a. Multiple Myelomatosis

Thus in the condition of multiple myeloma there may appear in the plasma large quantities of an abnormal globulin-like protein. The incorporation of ^{14}C-labelled amino acid into this protein follows an abnormal course, the isotope concentration both rising and declining very slowly (McFarlane, 1953). This result may be due, at least in part, to difficulty on the part of the body in the catabolism of the abnormal molecule.

[4] See McFarlane, *Brit. med. Bull.* 1952, 8, 213.—ED.

b. Nephrotic Syndrome

The nephrotic syndrome is characterized by a chronic loss of albumin and other plasma protein in the urine, and by low —often very low—values for circulating plasma albumin. Administration of labelled amino acid in a severe case of the syndrome results in an abnormal curve of incorporation of amino-acid nitrogen into plasma albumin. The curve rises and falls more steeply and reaches a higher peak than normal (Spector, 1954). Since the degree of abnormality apparently varies inversely with the level of circulating plasma albumin, this latter finding might suggest that the abnormal shape of the curves may be at least partly due to a reduction in the quantity of albumin available for labelling, rather than due to an increased rate of synthesis and increased rate of removal; that the enzymic reactions controlling albumin synthesis are not substantially altered in rate, and that the deficit due to loss of protein in the urine could be met by a reduction in the amount of protein broken down as a result of the process of catabolism. Such a reduction might follow from the diminished amount of albumin in the body. These interpretations are, of course, largely speculative. It is interesting, however, that Arnstein & Neuberger (1953) have concluded that the constant level of glycine in the normal rat is maintained by regulation not of the rate of synthesis but of the rate of degradation.

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DEMYELINATION

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In discussing the pathology of peripheral neuritis Kinnier Wilson (1940) has stated that " by whatever diverse and heterogeneous factors nerve substance sustains injury, its means of reaction are strictly limited and well-defined ". The series of changes involving the break-up and loss of the myelin sheath of the nerve fibre, either in peripheral nerves or in the central nervous system, constitutes one of the standard reactions to injury exhibited by nervous tissue, and has been described in a wide variety of different types of injury.

1. The Problem of Demyelination

The earliest account of the break-up of the myelin sheath was included in the classical description given by Waller (1850) of the gradual changes occurring in a peripheral nerve distal to a point of transection. Very similar changes in the myelin sheaths are seen in thiamine deficiency both in man (Pekelharing & Winkler, 1887) and in experimental animals such as the pigeon (Swank & Prados, 1942), and also in subacute combined degeneration of the cord (Greenfield & Carmichael, 1935). In lambs a deficient copper intake in the diet has been shown to cause extensive demyelination of the brain and spinal cord (Bennetts & Chapman, 1937; Bennetts & Beck, 1942).

Vascular damage with thrombosis, and anoxia due to other causes, provide examples of an injury of yet another type that can cause localized demyelination. The acute disseminated encephalomyelitis that can occur in the course of several of the common acute infectious diseases of childhood, and the demyelination of spinal tracts and of peripheral nerves caused by intoxication with certain organic chemicals, demonstrate this reaction of the nervous system in response to foreign toxic agents. And lastly, there is the large and important group of diseases of unknown origin, including disseminated sclerosis, which are collectively known as the " demyelinating diseases " of the nervous system.

It is probably true to say that demyelination can never exist, certainly for any considerable length of time, without some degree of damage to the axon. In some of the conditions mentioned above, however, and particularly in the group of the " demyelinating diseases ", damage to the myelin sheath in the majority of cases may exceed the degree of visible damage to the axons, and possibly may precede neurone damage. In other conditions, however, such as in thiamine deficiency, it would seem that neurone damage is primary (Swank & Prados, 1942).

To the pathologist, studies on demyelination have been largely orientated towards the problem of the origin and nature of the lesions of disseminated sclerosis and interpreted in terms of this problem, and many attempts have been made by different means to produce comparable lesions in experimental animals. Such attempts have largely failed, but although the types of lesion produced have often differed widely from those in disseminated sclerosis, this work has certainly been of value in throwing light on the normal metabolism of myelin and on the processes that can affect the state of the medullary sheaths. It is the purpose of this article to review briefly the various approaches that have been and are being made to the general problem of demyelination and the means by which nerve fibres can be made to undergo this reaction, rather than to review the more complex and distant problem of disseminated sclerosis.

The sequence of structural alterations that occur in the myelin sheath in the distal part of the nerve following section or crushing has been extensively described by earlier writers (Waller, 1850; Howell & Huber, 1892; Mönckeberg & Bethe, 1899; Ramon y Cajal, 1928; Nageotte, 1932), and recently the earlier changes have been re-examined by Weddell & Glees (1941). The last authors find in the rabbit that even though detectable alterations are visible in the axons 12 hours after section, definite changes in the myelin sheath are not apparent until some 12 hours subsequent to this; although Setterfield & Sutton (1935) could by means of polarized light discern changes even earlier than these, it is clear that demyelination in Wallerian degeneration is preceded by axonal changes. No gross alteration in the chemical character of the nerve can be detected in this early stage, however, and the process is presumably in the nature of a physical disintegration consequent upon loss of normal relationship with the intact axon.

By the beginning of the second week after nerve section chemical degradation of the myelin lipids (which consist principally of free cholesterol, cerebroside and sphingomyelin) becomes detectable and this proceeds rapidly till about the 32nd day (Brante, 1949; Johnson, McNabb & Rossiter, 1949, 1950). The latter authors suggest that the process of Wallerian degeneration, so far as the chemistry of the sheath is concerned, may be divided into three distinct stages. Stage 1 comprises the first 8 days, during which time there is a physical break-up unaccompanied by any gross chemical degradation. Stage 2 is found from the 8th to the 32nd day and corresponds to the " Marchi stage " of Spielmeyer (1929) and the beginning of his " Scharlach-red stage ". During this phase there is a marked fall in the concentration of the myelin lipids accompanied by a rise in that of cholesterol esters. Stage 3 represents the state of affairs after the removal of the myelin and is characterized by increasing amounts of collagen (Abercrombie & Johnson, 1946a). These chemical studies correlate well, on the one hand with the histochemical findings of Nobak & Montagna (1952) and, on the other, with the changes in the cell populations of the degenerating

nerve. Nuclear counts were made by Abercrombie & Johnson (1946b) at different times and yielded the information that cell proliferation was well under way by the 5th day, that by the 15th day a 9-fold increase had occurred and that this increase reached a peak by the 25th day, falling off thereafter. The great majority of the cells were Schwann cells and macrophages, differentiation between these not being sufficiently easy to allow estimation of their relative proportions. It is clear from these observations that the chemical breakdown of the myelin is closely associated with cell activity, and morphologically there is good reason for believing that the macrophages are almost the sole agents in this respect (Young, 1942). Moreover, that the fragmenting myelin is the specific stimulus for such cellular activity is indicated by the extremely poor cellular response that occurs in degenerating unmyelinated nerve (Joseph, 1947). On turning to the central nervous system, it is found that in all essentials the break-up of the myelin in tract degeneration passes through the same stages as in peripheral nerve, except that glial cell activity replaces that of the Schwann cell and macrophage (Ramon y Cajal, 1928).

Although Wallerian degeneration is a pathological process which has probably been studied more than any other affecting the nervous system, it must be apparent that these cytological changes are purely secondary phenomena, and provide no clue to the initiating lesion that may destroy either the axon or possibly the myelin sheath.

The nucleic acids and protein-bound phosphorus levels in nerve have also been studied during Wallerian degeneration (Bodian & Dziewiatkowski, 1950; Samuels, Boyarsky, Gerard, Libet & Brust, 1951; Logan, Mannell & Rossiter, 1952). Total nucleic acids increase rapidly after nerve section or nerve crush, reaching a maximum at about 16 days, falling thereafter slowly but remaining above the normal level for a long time in the regenerating nerve. This rise in nucleic acid content is probably largely due to the cellular increase noted above. According to Logan *et al.* (1952) the concentration of pentosenucleic acids increases more than that of the deoxypentosenucleic acids. Although the total protein-bound phosphorus increases in degenerating nerve, this is due to the increase in nucleic acid phosphorus, and the amount of phosphoprotein appears actually to fall.

As regards the enzyme content of degenerating nerve, it has been shown by Sawyer (1946) that, after section of the guinea-pig sciatic nerve, pseudo-cholinesterase activity remains largely unaffected, although a substantial reduction of the true cholinesterase occurs. Choline acetylase activity also diminishes (Nachmansohn, John & Berman, 1946), and von Muralt & von Schulthess (1944) have described a fall in acetylcholine content. Esterase activity (with methyl butyrate as substrate) has been described as falling to about 40% of the level in normal nerve (Lumsden, 1952). β-Glucuronidase activity, on the other hand, undergoes a 30- to 40-fold increase in cat sciatic nerve 16 days after nerve section (Hollinger, Logan, Mannell & Rossiter, 1952), and Heinzen (1947) has reported that acid phosphatase activity is also increased. The thiamine content of degenerating nerve is said to be decreased (von Muralt & Wyss, 1944).

These findings, and in particular the more recent chemical data, have been largely obtained, as will have been noted, from nerve tissue undergoing the classical Wallerian degeneration which follows nerve section. We are therefore faced with the question as to how a similar histological picture develops in the variety of conditions that present themselves in clinical neurology, and with the problem of the immediate changes and causes which lead up to this picture of myelin loss. Some light has been thrown on possible causes, both by veterinary observations and by experimental work in laboratory animals.

2. Copper Deficiency

Enzootic ataxia, first described in detail by Bennetts (1932), is a demyelinating disease of young lambs occurring in Australia but related to similar conditions described in other countries. Because the disease appeared to be confined to animals on certain "affected" pastures, and because of the absence of any evidence of an infective aetiology, the condition was thought to be probably of nutritional origin, and evidence was soon forthcoming to demonstrate an associated copper deficiency. Thus, the copper content of the livers of the ataxic lambs was low, as were also the copper levels in the blood and milk of gestating ewes grazing on "affected" pastures (Bennetts & Chapman, 1937). Bennetts & Beck (1942) further showed that these pastures were themselves deficient in copper. The course of the ataxia in lambs could be arrested by administration of copper, while copper therapy of pregnant ewes on the deficient pastures afforded complete prevention of the condition in the lambs.

Swayback is a condition clinically resembling enzootic ataxia that affects lambs in certain parts of Great Britain. It is characterized by a diffuse symmetrical cerebral demyelination with additional degeneration of the motor pathways in the spinal cord. The lesions vary from microscopic changes to gross damage with liquefaction and cavitation (Innes, 1939). In this condition, however, the relationship to copper deficiency is not so clear; for example, the pasture in the swayback areas is not low in copper content. Despite this, however, it has been shown that the copper levels in the blood and liver of ewes grazing in these areas and of ataxic lambs are consistently below normal (Innes & Shearer, 1940). The blood-copper level could be raised to normal by transferring the ewes early in pregnancy to pastures where the disease had not occurred (Eden, 1944; Shearer & McDougall, 1944), and, as with enzootic ataxia, it was found that swayback could be prevented by the administration of copper (Dunlop & Wells, 1938; Dunlop, Innes, Shearer & Wells, 1939).

From these studies it is clear that in the lamb an association exists between copper deficiency and a destruction or failure of formation of myelin. Since the ataxia is present only in lambs and not in grown sheep the latter would seem to be more likely. In view of these findings blood-copper and urine-copper levels were examined in a series of 26 cases of disseminated sclerosis (Mandelbrote, Stanier, Thompson & Thruston, 1948), but no evidence of any abnormality of copper metabolism was disclosed in the cases studied. Disseminated sclerosis is indeed very different in its natural history from swayback of lambs, but a more closely analogous human condition exists in one variety of porencephaly in infancy, as noted by Lumsden (1950a). The results of a study of the copper metabolism in this condition would be of interest.

3. Cerebral Anoxia

To turn now from a naturally occurring disease of livestock to the attempts to reproduce demyelinating conditions experimentally, the effect on the brain of anoxia produced by

cyanide and other chemical substances has been fairly extensively studied. Ferraro (1933a, 1933b) succeeded in producing disseminated foci of demyelination in brains of cats and monkeys by administration of potassium cyanide. Hurst (1940, 1941, 1942) studied the condition further and demonstrated that size of dose was an important factor. Large doses of potassium cyanide produced cortical damage, with smaller doses the basal ganglia were predominantly attacked, while with repeated still smaller doses focal lesions in the white matter resulted. These lesions affected both myelin sheaths and axis cylinders and were akin, with slight differences in detail, to those produced by sodium azide, carbon monoxide (Meyer, 1928; Putnam, McKenna & Morrison, 1939; Yant, Chornyak, Schrenk, Patty & Sawyers, 1934) and other anoxia-producing agents. Two observations—that the lesions of cyanide poisoning do not appear to be necessarily located around blood vessels and that there is some persistence of axons within the demyelinated areas (Lumsden, 1950b)—indicate that a fairly close resemblance to the plaques of disseminated sclerosis obtains.

It is, however, not yet clear whether the loss of myelin induced by these agents is the direct outcome of inhibition by them of the cytochrome-cytochrome oxidase mechanism in the central nervous system, since Wyndham (1941) was unable to find any over-all diminution in the oxidation mechanisms of the white matter of monkeys poisoned with cyanide.

A local anoxic state resulting from thrombotic occlusion of blood vessels has been held by Putnam (1936, 1937) to be causal in disseminated sclerosis. Dow & Berglund (1942) have shown, however, that only about two-thirds of the plaques in this disease are related to vessels, and that thrombosis when it occurs is probably the result rather than the cause of the lesion. This opinion agrees with earlier authors (for example, Dawson, 1915) who held that, although the toxic agent probably reached the brain through vascular channels, local vascular changes were secondary manifestations. It has in fact never been possible by micro-embolism to produce changes really closely resembling those of disseminated sclerosis in the same way as do the cyanide lesions.

The reason why the myelin sheath, rather than the reputedly much more sensitive neurone and axis cylinder, suffers under these conditions of prolonged partial anoxia is not very clear. It may be that the white matter, with its poorer capillary anastomoses and blood supply and its lower rate of respiration, has less recuperative power than the grey, and so suffers more from repeated minor insults of partial anoxia.

4. "Allergic" Demyelination

A considerable number of clinical and experimental observations have been adduced in favour of a hypothesis that some " allergic " response may constitute the basis of certain forms of demyelinating disease. In the clinical field the acute encephalomyelitis that can complicate vaccinia and a number of the acute infections of childhood could be interpreted in support of such a view, and it was suggested that disseminated sclerosis itself might be an allergic reaction to some unknown abnormal protein, although there is little evidence to support this view.

It is certainly now established beyond doubt that a so-called " allergic " demyelinating encephalopathy can be produced in experimental animals by non-infectious means. Thus Rivers & Schwentker (1935) produced demyelinating lesions in the brain of monkeys by the injection of sterile extracts of normal rabbit brain, and their findings were confirmed by Ferraro & Jervis (1940), Kabat, Wolf & Bezer (1947), Morgan (1947) and Lumsden (1949a, 1949b). However, from the point of view of the underlying biochemical changes occurring in the myelin, this work, apart from affording a further means of producing demyelination experimentally, has not shed much light on the problem of the immediate causes of myelin break-up.

5. Demyelination due to the Action of Anticholinesterases

In recent years it has been found that certain of the anticholinesterases can cause demyelinating lesions in the nervous system. The compound that has been most extensively studied from this point of view is tri-*o*-cresyl phosphate, the substance which was shown to be the causative agent of a wide-spread outbreak of motor neuritis in the United States of America (Smith & Elvove, 1930; Smith, Elvove & Frazier, 1930). The paralysis is associated with lesions in the peripheral nerves and in certain tracts of the spinal cord. Smith & Lillie (1931) considered that the compound was a " specific myelin poison ", but it appears from our own preliminary studies of hens poisoned by this substance that the axons are sufficiently damaged to account for the degenerative changes in the myelin sheath, and that the myelin changes are of the Wallerian type. Moreover, degenerative changes appear to be more pronounced at the distal ends of the nerve fibre and to spread centrally with the course of time, and also large fibres appear more susceptible than smaller-sized fibres. In the bird, the ventral tract is affected principally in the lumbar region, the spinocerebellar tracts and to a lesser degree the posterior columns are damaged in the cervical region, while in the brain stem the large decussating fibres of the vestibulocochlear system may also be involved. The selection of the tracts and the general distribution of the changes strikingly resemble the systematized lesions in experimental thiamine deficiency in the bird (Bertrand, Liber & Randoin, 1934; Swank & Prados, 1942), and although an interference with the metabolism of myelin may still possibly be the initiating factor it would appear more likely that the metabolic processes of the neurone are in some way disturbed by the tri-*o*-cresyl phosphate.

Tri-*o*-cresyl phosphate is, however, a selective inhibitor of pseudo-cholinesterase (Mendel & Rudney, 1944; Earl & Thompson, 1952a)[1], and in hens poisoned by a single oral dose of this compound Earl & Thompson (1952b) found that the level of pseudo-cholinesterase activity in the brain and spinal cord is very markedly inhibited one day after poisoning and remains low for at least ten days; the true cholinesterase in these tissues was unaffected by the dosage used, which regularly causes profound weakness and ataxia in about 14 days in hens. It had earlier been shown that pseudo-cholinesterase is chiefly associated with the white matter of the central nervous system (Ord & Thompson, 1952), and Koelle (1951) has demonstrated its presence histochemically in the glial cells of sensory and autonomic ganglia and in the Schwann cells of myelinated nerves. In human peripheral nerve Thompson & Webster (in preparation) have shown that pseudo-cholinesterase is present in significantly greater amounts than the true cholinesterase, while an oligodendroglioma examined by them contained a higher activity than

[1] See also Thompson, *Brit. med. Bull.* 1953, 9, 138.—Ed.

any other area of the human nervous system so far studied, although exhibiting only traces of true cholinesterase. It seemed possible therefore that pseudo-cholinesterase in the nervous system might be concerned with some aspect of myelin formation or turnover, and that its inhibition by tri-*o*-cresyl phosphate might be causally associated with the ensuing demyelination.

If this were so, it would be expected that poisoning with other selective inhibitors of pseudo-cholinesterase would also lead to loss of myelin and paralysis, and this has now been shown to be the case for two other anticholinesterases. With one of them the first indication was obtained from a clinical report of two cases of poisoning (Bidstrup & Hunter, 1952; Bidstrup, Bonnell & Beckett, 1953); after occupational exposure to bis(*iso*propylamino)fluorophosphine oxide the two patients developed a flaccid paralysis which was said to resemble clinically that which followed poisoning by tri-*o*-cresyl phosphate. Barnes & Denz (1953) next succeeded in producing demyelination and paralysis in hens both by this compound and by di-*iso*propyl fluorophosphonate (DFP). On the other hand, poisoning by certain other inhibitors of pseudo-cholinesterase has not resulted in demyelination, even although a marked inhibition of this enzyme has been produced in the nervous system by them. If, therefore, inactivation of pseudo-cholinesterase in the nervous system is causally connected with the development of the demyelination, it cannot be the only factor in these cases. It is possible that the demyelination may result from some other action. But as regards tri-*o*-cresyl phosphate, inhibition of pseudo-cholinesterase and tributyrinase are the only biochemical effects which have so far been described (Hottinger & Bloch, 1943). Moreover, of these two enzymes in brain, tributyrinase is very much less sensitive to inhibition by tri-*o*-cresyl phosphate, while glucose and pyruvate oxidation by brain, amine oxidase, trypsin, lecithinase and cephalinase are all unaffected even by high concentrations (Earl, Thompson & Webster, 1953). Hottinger & Bloch (1943) had earlier shown that serum alkaline phosphatase and pancreatic lipase are also insensitive. The mode of action of these anticholinesterases in the production of demyelination is therefore obscure. It is of interest, however, in this connexion to recall that one of the early theories put forward to account for the development of disseminated sclerosis postulated the action of some "myelinolytic ferment" (Marburg, 1906). Several later attempts were made to demonstrate the presence of excessive amounts of an esterase or lipase in the plasma and urine which, it was held, might be capable of myelinolysis (Brickner, 1930; Crandall & Cherry, 1931; Weil & Heilbrunn, 1941). Much of this early work is difficult to assess on account of the technical methods then in use, but Richards & Wolff (1940), using manometric methods, were unable to detect any increase in the esterase, lipase or cholinesterase contents of the serum from patients with disseminated sclerosis. Vogel (1950), on the other hand, has claimed to produce scattered areas of demyelination, resembling the plaques of disseminated sclerosis, by the intracerebral injection of purified pancreatic lipase.

6. Discussion

It will be apparent from this brief survey of the various means by which demyelination can be brought about experimentally that not only is it not yet possible to reproduce in laboratory animals the true counterpart of the lesions of disseminated sclerosis, but also that we have as yet no clear idea as to the underlying biochemical mechanism in most of the experimental demyelinations.

From the biochemical point of view four apparently dissimilar procedures can give rise to these lesions: (i) copper deficiency, (ii) intoxication by cyanide, presumably acting by producing cerebral anoxia, (iii) intoxication by certain of the anticholinesterases and (iv) deficiency of certain vitamins of the B complex, notably thiamine and vitamin B_{12}. It must be stressed, however, that although the individual lesions are similar in these four conditions, their localization differs widely.

One important factor which has caused some difficulty and confusion in past studies of experimental deficiency neuropathies, and which may ultimately shed some light on the normal metabolic processes of the myelin sheath, is the effect of starvation alone in producing fragmentation and positive Marchi staining of the sheath even when thiamine is administered to the animals (Woollard, 1927; Prickett, 1934; Prickett, Salmon & Schrader, 1939; Engel & Phillips, 1938). According to Swank (1940) these changes are, however, distinguishable from the Wallerian degeneration occurring in the vitamin neuropathies not only by the appearance of the lipoid granules formed from the sheath, but also by the absence of any changes in the axis cylinder. Further difficulties in the interpretation of experimental results, and also in the understanding of the metabolic background on which the deficiency state acts, are due to variation in sensitivity of different species to vitamin deprivation; and no evidence has yet been forthcoming to account, for instance, for the notorious resistance of the rat to thiamine deficiency, that contrasts with the sensitivity of the avian species. Again, other problems concerning the metabolism of the nerve fibre and its sheath are encountered when thiamine deficiency in the pigeon is studied. Swank (1940) showed that acute complete deprivation of the vitamin resulted in severe opisthotonos and death of the bird without the development in many instances of peripheral neuropathy; this latter state was found to be produced by chronic partial deprivation. Although the factor of time may be invoked to account for these findings, the general picture suggests that there is possibly some ill-understood physiological mechanism connected with the metabolism of the myelin sheath and the axis cylinder that only manifests itself in morphological changes when working under inadequate conditions of energy supply.

For a further analysis of the mechanisms underlying these various types of lesion it would be desirable to know more about the relative roles played by the neurone and the glial or Schwann cells respectively. It has been widely held that the oligodendrocyte centrally (del Rio Hortega, 1921, 1928) and the Schwann cells peripherally are in some way concerned with the maintenance of the myelin sheaths. On the other hand, we know from the effects of nerve section that continuity of the axon with the perikaryon is essential for the existence of the myelin sheath, although it must be admitted that practically nothing is known of the influence of the axon upon its sheath under normal circumstances. From the nature of the biochemical lesion in thiamine deficiency (Peters, 1936)[1], however, it might be expected that the associated initial myelin changes are secondary to the block in the energy-yielding reactions of the neurone resulting from the inhibition of pyruvate oxidation. Further, since raised blood pyruvate

[1] See also Peters, *Brit. med. Bull.* 1953, **9**, 116.—ED.

levels have recently been reported in untreated cases of subacute combined degeneration of the cord (Earl, El Hawary, Thompson & Webster, 1953) and since the levels are rapidly restored to normal by treatment with vitamin B_{12}, it may be that the demyelination in this condition is also secondary to a block in the carbohydrate metabolism of the nerve cells. In this connexion it is relevant to recall the closeness with which these two human diseases resemble one another; in the cord the long tracts bearing large heavily myelinated fibres are particularly selected in both conditions, while the same large fibres are principally involved in the peripheral nerves. In the case of the demyelination due to selective inhibitors of pseudo-cholinesterase, however, particularly as this enzyme is certainly present in relatively large amounts in glial cells, the role of the oligodendrocyte, and possibly of the astrocyte, may well be important. On the other hand, while it might be considered that a similar block in the energy-yielding enzyme systems of the neurones and glial cells might also be operative in producing the lesions due to cyanide, the striking differences in the mode of onset and distribution of these lesions compared with those of thiamine deficiency would seem to support the view that other factors may be responsible.

One further fact would seem to be emerging from the more recent work. Many of the earlier workers on these problems postulated an accelerated breakdown of myelin, which they called myelinolysis. It is now known, however, from isotopic studies, that myelin is in a constant state of " turnover ", with new formation balancing its disintegration, and it is certainly possible that the complex series of synthetic reactions involved in this formation of myelin may become deranged. Indeed on a priori grounds it would seem more likely that the demyelination in anoxia or in thiamine deficiency would be due to a failure in endothermic synthetic processes.

Lastly, there is the further problem, common to so many neurological disorders, of the localization of demyelination to certain tracts or to scattered plaques within the nervous system. In the tract degeneration, associated so frequently with peripheral neuritis, the distance separating the terminal parts of the long axons from their cell bodies would seem to be operative, suggesting again that these lesions are due to some decline in the controlling or nutritional influence of the perikaryon. But other factors again must certainly operate in the production of the scattered, discrete localized lesions of disseminated sclerosis.

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GAS GANGRENE

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1 Definition
2 The infecting organisms
3 Genesis of the infection
4 Colonization of injured and uninjured muscle
5 Prophylaxis and treatment
References

1. Definition

Gas gangrene is a rapidly-spreading oedematous myonecrosis, occurring characteristically in association with severe wounds of extensive muscle masses that have become contaminated with pathogenic spore-bearing anaerobes, particularly with *Clostridium welchii*. Muscle so infected, the blood supply of which has been wholly or partly cut off, is especially liable to gas gangrene. In inadequately treated cases toxaemia is profound, a haemolytic anaemia is the rule, and circulatory failure is common. The host reaction in muscle undergoing gas gangrene is negligible; in fact, all the local changes occurring in gas gangrene due to *Cl. welchii*, except oedema and gas formation, can be produced in sections of normal muscle by incubating them with sterile culture-filtrates of the organisms. The term "clostridial myositis" is therefore a misnomer, but it is so embedded in the literature that its replacement by "clostridial myonecrosis" is hardly likely (Robb-Smith, 1945 ; Power, 1945b).

This definition of gas gangrene explicitly excludes the much more benign anaerobic cellulitis, in which the spread of anaerobic organisms is limited to the subcutaneous tissues, toxaemia is absent and gas formation is far greater than it is in anaerobic myonecrosis (MacLennan, 1943a ; Lowry & Curtis, 1947). Similarly myositis due to anaerobic streptococci is excluded; in that condition muscle involvement is usually focal, the discharge is usually far more purulent than it is in clostridial myonecrosis, toxaemia is inconstant and surgical treatment is to be avoided (MacLennan, 1943a, 1943b; Lowry & Curtis, 1947).

Gas gangrene as thus defined is a disease of war in which extensive wounds of muscle are produced by shell or bomb splinters, or by mines, under conditions in which the wounds are readily contaminated with pathogenic anaerobes. Similar injuries may occur in particular trades, such as, for instance, whaling, in which severe injuries from loose tackle are common, and the decks of the ships are heavily coated with whale faeces, in which pathogenic clostridia abound (Case, 1948; Robinson, Ingram, Case, Benstead & Daniels, 1953). Before the introduction of antiseptic surgery, gas gangrene was common in persons in whom muscle damage had been produced by surgical intervention; nowadays the only common civil source of gas gangrene—apart from motor accidents—seems to be septic abortion, in which the area of damaged muscle and other tissue is provided by the injured placental site, and the clostridia are introduced with the abortifacient (Butler, 1943, 1945; Ramsay, 1949).

2. The Infecting Organisms

Obviously the chance of clostridial infection of a wound will depend on the concentration of clostridia in the environment, so it is not surprising that the incidence of gas gangrene varied greatly in different theatres of war. Thus, in fighting in the desert, gas gangrene was rare; the hot desert sand, even in areas soiled by human occupation, harbours few anaerobes, and though *Cl. septicum* and *Cl. sporogenes* spread on exposed desert sand, either as culture or as infected faeces, survived for at least 12 weeks, *Cl. welchii* under the same conditions survived one week only. In cultivated areas and near roads *Cl. welchii* and *Cl. sporogenes* were common and gas gangrene occurred more frequently in wounds sustained in such areas. But though anaerobes occurred so infrequently in desert fighting, gas gangrene did occur in wounds sustained there, and this drew MacLennan's attention to the frequency with which men's immediate environment—their clothes—was contaminated with pathogenic anaerobes; both battle-

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dress and uniforms made of khaki drill yielded clostridia on culture (MacLennan, 1943c). Fragments of such clothing driven into severe wounds might easily provide sufficient clostridia to infect them, even though the surrounding ground was almost sterile. This may also account for the 18 cases of gas gangrene reported by Cutler & Sandusky (1944) in 1,000 wounds suffered in aerial combat.

Cultures of muscle from cases of gas gangrene seldom yield a single clostridial species in pure culture; almost invariably several species are present. Sometimes all are pathogenic: *Cl. welchii, Cl. oedematiens, Cl. septicum.* Sometimes one or other of these is associated with non-pathogenic clostridia, with *Cl. butyricum, Cl. tertium* and their allies or, more important, with proteolytic forms such as *Cl. sporogenes* or *Cl. histolyticum*; or even with streptococci or *Bacterium coli* (MacLennan, 1943c ; Zeissler, 1946).

It seems doubtful whether clostridia other than *Cl. welchii, Cl. septicum* and *Cl. oedematiens* are capable of producing gas gangrene by themselves; what is more probable is that proteolytic forms such as *Cl. histolyticum* and *Cl. sporogenes* multiply in the necrosed tissue produced by the three common pathogenic forms, and by breaking it down provide nutriment for themselves, for other anaerobes, and for the heterogeneous collection of other organisms that may occur in war wounds. Certainly the presence of *Cl. histolyticum* greatly increases the mortality in gas gangrene due to other anaerobes (MacLennan, 1943b).

Anaerobic infection of wounds is, however, far more common than gas gangrene. MacLennan (1943c) found that anaerobes were present in from 18% to 40% of all war wounds, whereas gas gangrene developed in only 0.32% of such wounds; Cutler & Sandusky (1944) found that, though 24% of wounds were infected with clostridia, only 1.8% developed gas gangrene; Stock (1947) isolated clostridia (usually *Cl. welchii* or *Cl. oedematiens*, sometimes both) from all of a small number of wounds, whether they developed gas gangrene or not. The clostridia isolated from wounds that did not develop gas gangrene were very similar both in identity and in frequency of occurrence to those isolated from gangrenous muscle. Evidently more is required for the development of gas gangrene than the presence of the infecting clostridia.

3. Genesis of the Infection

All the important organisms that occur in cases of gas gangrene are strict anaerobes; that is, they are unable to grow or reproduce in even moderate concentrations of oxygen or, for that matter, in well-oxygenated tissues.[1] The state of oxygenation of fluids is best expressed as the oxidation-reduction potential (E_h); high values indicate that the fluid will readily give up oxygen, low ones that it will give up oxygen with difficulty, negative ones that it will absorb oxygen with more or less readiness. The E_h of circulating blood varies between + 0.126 v and + 0.246 v, with most of the figures lying between + 0.156 v and + 0.186 v (Hanke & Tuta, 1928), and it seems reasonable to suppose that the E_h of the tissues does not differ greatly from these figures. *Cl. welchii*, which is not a very strict anaerobe, requires at the pH of the tissues an E_h lower than + 0.074 v before it can grow; most anaerobes are much stricter in their requirements and, though they would no doubt survive in normal tissue, would certainly be unable to grow or reproduce there.

[1] See Payling Wright, p. 59 of this number of the Bulletin.—ED.

This state of affairs is entirely altered by destructive injuries to muscles. Besides the direct injury they produce, penetrating bomb or shell fragments may, by damaging large arteries, cut part or all of the blood supply of the muscle; and, as Le Gros Clark (1945) has shown in rabbits, the presence of an anastomotic blood supply is insufficient to prevent necrosis of the muscle; revascularization is delayed too long (Power, 1945a). Vascular damage is of great importance in predisposing to gas gasgrene; according to North (1947), 72% of cases of gas gangrene have injuries to the vessels supplying the affected muscle; Lowry & Curtis (1947) found that, out of 108 amputations for gas gangrene, 97 had gross vascular damage.

To the direct injuries produced by shell or bomb fragments or mine explosions are added those due to the explosion waves that penetrating missiles set up in the tissues. These lead to tearing and cavity formation, so that vessels are torn through or go into spasm and bones are fractured well away from the path of the missile (Harvey & McMillen, 1947). Moreover, as the hole by which the missile enters the muscle is relatively small compared with the mass of damaged muscle beyond it, drainage of extravasated blood may be hindered, the pressure in the damaged muscle may rise, and the capillaries may be seriously compressed.

But, taken together, all the injuries lead to the same results: to the development of areas of anoxic muscle and to more or less extensive haemorrhages. In anoxic muscle, breakdown of carbohydrate continues and the oxidation-reduction potential falls. The haemoglobin in the extravasated blood is rapidly reduced, and ceases to act as an oxygen carrier; the myohaemoglobin in the damaged muscle behaves in the same way; aerobic oxidation cannot be carried out, and anaerobic reduction of pyruvate to lactate takes its place, with consequent drop in E_h.

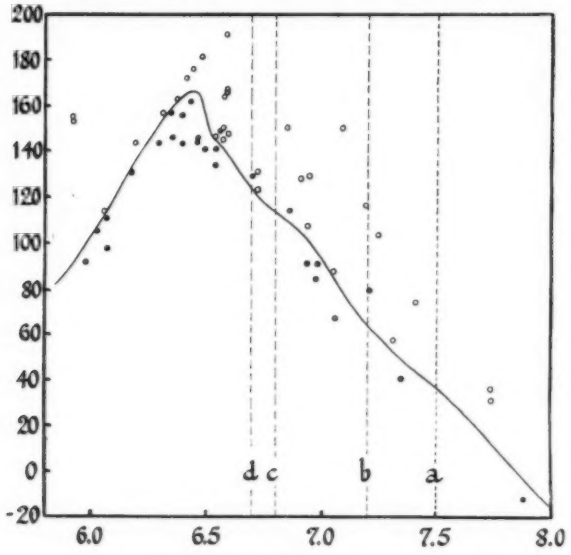
As lactate accumulates in the muscle, alkali reserve is used up and the pH falls (Zamecnik, Aub, Brues, Kety, Nathanson, Nutt & Pope, 1945), and, as a consequence of the change in E_h and pH, conditions become more favourable for the proteolytic enzymes present in muscle; these are activated and break down some protein with increase of amino acids and further fall in pH.

This increase in the concentration of amino acids favours the growth of clostridia in two different ways: it provides them with food material, and it raises the level of E_h at which they can grow. Figure 1 shows that, as the pH of the medium falls, so the maximum E_h at which *Cl. welchii* will grow tends to rise until it reaches a maximum level at pH 6.4 (Hanke & Bailey, 1945). As Stoner & Green (1948) have shown, two hours after interruption of the blood supply the pH of anoxic muscle has fallen to 6.8; at this level *Cl. welchii* would grow at an E_h of about + 0.115 v, a figure slightly below that of normal blood, but one that could readily be reached in anoxic muscle if a small amount of anaerobic glycolysis had occurred (Smith, 1949).

The conditions within damaged muscle are therefore ideal for the development of anaerobes, particularly for the less strict anaerobes, e.g. *Cl. welchii*, and the products of muscle breakdown provide them with excellent nutriment. In the early stages of muscle injury, protein appears to leak into the damaged muscle from the capillaries (Zamecnik *et al.* 1945; MacLennan & Macfarlane, 1945), but it is evident that in the later stages the conditions in anoxic muscle—vascular damage, extensive haemorrhage, thrombosis—will not favour

FIG. 1. RELATIONSHIP BETWEEN pH OF CULTURE AND MAXIMUM E_h AT WHICH *CLOSTRIDIUM WELCHII* WILL GROW

(Curve constructed from the tables of Hanke & Bailey, 1945; pH values of normal and anoxic muscle from Stoner & Green, 1948)



Abscissae : pH of culture
Ordinates : E_h of culture (mV.)

• growth
o no growth

Dotted lines
- a: pH of normal muscle
- b: pH of muscle anoxic for 1 hr.
- c: pH of muscle anoxic for 2–3 hr.
- d: pH of muscle anoxic for 4 hr.

the ordinary defences of the body. Polymorphs cannot readily enter from the damaged vessels and, as they are strict aerobes, cannot penetrate far into the anoxic tissues (cf. the leucocytic ring in transplants of liver (Cameron & Oakley, 1934)), while antibodies and other substances important in humoral defence cannot enter the swollen devitalized muscle.

4. Colonization of Injured and Uninjured Muscle

The success of anaerobes in colonizing first the damaged and later the undamaged muscle seems largely to be due to the exotoxins they produce. It is convenient for the present to limit ourselves to *Cl. welchii* type A in this respect, as far more is known about it than about most other anaerobes. *Cl. welchii* produces a lecithinase (α-toxin), which breaks down lecithin into phosphorylcholine and stearyloleylglyceride (Macfarlane & Knight, 1941); as lecithin occurs in all cell membranes (though it is not always easily attackable by lecithinases when it is present: see Oakley, Warrack & Clarke, 1947; Macfarlane, 1950), organisms secreting lecithinases are often capable of damaging or destroying cell-membranes, and thus of seriously interfering with the metabolism of the cells. Thus, by its action on capillaries, α-toxin may render them freely permeable to fluid and protein, and so increase the tension in the affected muscle and render it still more anoxic. The α-toxin of *Cl. welchii* is, in suitable concentrations, lethal to whole animals and to groups of animal cells and will haemolyse the red cells of most laboratory animals (but not horses and goats) and of man (Oakley, 1943). By the breakdown of lecithin and sphingomyelin, which it attacks rather more slowly (Macfarlane, 1942, 1948), it provides lipid as nutriment for the organisms, and opens up more cells to their attack. As O'Meara (1945) has pointed out, *Cl. welchii* produces far more α-toxin in media containing cooked meat particles, and would presumably be expected to produce large amounts of the toxin in gangrenous muscle. A haemolytic anaemia is the rule in *Cl. welchii* gas gangrene, and this is usually supposed to be due to α-toxin.

Intravenous injection of *Cl. welchii* filtrate into guinea-pigs produces necroses in the liver (Weinberg & Combiesco, 1930—but these hepatic necroses may not be due to α-toxin) and might be expected to facilitate metabolic growth of *Cl. welchii* in those cases in which a bacteraemia occurs. Intramuscular injections of *Cl. welchii* filtrate into dogs apparently produce, besides oedema, haemorrhage and muscle necrosis at the injection site, and severe changes in the kidneys: in three to four hours the kidneys show glomerular swelling, and swelling and nuclear changes in the proximal convoluted tubules and in the Henle's loops. Encasing the injected area in plaster appears to reduce the incidence of renal damage; massage of the injected area seems to increase haemolysis, so that haemoglobin appears in the renal tubules (Berg, Levinsen & Wang, 1951). In neither case is any evidence provided that the lesions are due to α-toxin, but this seems not unlikely (cf. Zamecnik, Nathanson & Aub, 1947).

The other haemolysin produced by *Cl. welchii*, θ-toxin, is an oxygen-labile lysin, active only under reducing conditions, apparently antigenically related to other oxygen-labile lysins (Todd, 1941). It is probably a leucocidin (Todd, 1942) but, as it is not usually produced in media containing meat particles, or, if produced, is absorbed by them (Schnayerson & Samuels, 1930), it seems improbable that it will be of importance in gangrenous muscle. Besides this possible leucocidin, ν-toxin (deoxyribonuclease) breaks down the deoxyribonucleic acid of cells, and particularly of leucocytes; whether it is able to kill living leucocytes is uncertain (Oakley & Warrack, 1951).

No less important in assisting the spread of *Cl. welchii* type A in the tissues are the collagenase (κ-toxin) and the hyaluronidase (μ-toxin). Collagenase breaks down collagen and reticulin, without affecting elastin, both in the intact animal and in vitro, and in consequence destroys collagen barriers to the spread of *Cl. welchii*, while the destruction of reticulin around capillaries leads to haemorrhage and thrombosis, with further interference with the blood supply of the muscle (Macfarlane & MacLennan, 1945; Oakley, Warrack & van Heyningen, 1946; Oakley, Warrack & Warren, 1948). From the broken-down collagen and reticulin further food supplies in the organisms are obtained, while the destruction of the reticulin and collagen scaffolding leads to disintegration of the muscle, separation of its fibres, and a more complete exposure to the proteolytic effect of the muscle enzymes and those of accompanying proteolytic anaerobes. Hyaluronidase breaks down hyaluronic acid, a polysaccharide present in most tissues, particularly in skin and vitreous humour, which acts as a physical obstruction to the spread of bacteria and of their toxins (McClean, 1936).

As would be expected, hyaluronidase-producing organisms spread much more readily in the tissues than organisms that do not produce it; and it can readily be shown that addition of hyaluronidase to cultures of *Cl. welchii* that do not produce it greatly increases the rapidity with which they spread through the tissues of infected animals. But the possession of hyaluronidase does not seem to determine virulence to any great extent (McClean & Rogers, 1943); and Dalgaard-Mikkelsen, Karlshøj & Szabo (1950) consider that its importance to the organism is rather to provide it with supplies of fermentable sugar from the breakdown of hyaluronic acid than to assist its physical spread. (But see Haas (1946a, 1946b) on the importance of hyaluronidase in bacterial invasion.) Other sources of fermentable carbohydrate are no doubt provided by enzymic breakdown of group A (Schiff, 1939) and possibly group O blood-group substance (for action of type B strains of *Cl. welchii*, see Stack & Morgan, 1949). Whether the mucopolysaccharase of Werle & Bauereis (1942) is of importance is uncertain; normal human sera do not inhibit it.

As Robb-Smith (1945) has stated, almost all the changes seen in muscle in gas gangrene, with the exception of the obviously vital changes (oedema, gas formation) can be reproduced in vitro by the action of *Cl. welchii* filtrates on thin tissue slices; but it is only fair to point out that some authors have not been convinced that this ideal comparison between enzyme activity in vitro and the damage produced in the organism is the whole truth. For instance the importance of collagenase, θ-toxin and hyaluronidase has been called into question by Evans (1943, 1947). He found that, in guinea-pigs in which gas gangrene had been produced by intramuscular injection of calcium chloride and subsequent infection with *Cl. welchii*, antisera containing *Cl. welchii* α-antitoxin would save the animals from death, whether injected before the infecting dose or a few hours after; θ-antitoxin, anti-hyaluronidase and anti-collagenase were not only ineffective alone, but they did not improve the effect of α-antitoxin, if they were injected with it. The natural conclusion from this is that α-toxin only is of any serious importance in the development and spread of gas gangrene; but against this two objections can be made. The first is that the disease in guinea-pigs differs somewhat from the disease in man, particularly in the fact that the muscle masses available for infection are relatively thin, so that extension outside them occurs very readily; the second is that, though α-toxin may be necessary to initiate the process of bacterial infection, which may indeed not occur without it, once α-toxin has started the process the other toxins may contribute notably to its development. Evans (1945) did indeed find some relationship between collagenase production and lecithinase production on the one hand, and virulence for guinea-pigs on the other; other authors (Keppie & Robertson, 1944) found little relation between pathogenicity for guinea-pigs and α-toxin or hyaluronidase production, or the formation of capsules. The last point had been stressed by Butler (1945), who claimed that capsule production in *Cl. welchii* and resistance to phagocytosis appear to go hand in hand; but in most cases of gas gangrene phagocytosis is reduced or abolished by conditions depending far more on the environment than on the capsulation of the infecting organism.

A more formidable objection to the views of Robb-Smith (1945) is that raised by Macfarlane & MacLennan (1945), who have suggested that the toxaemia and anaemia so commonly seen in severe cases of gas gangrene are due, not to α-toxin, but to some toxic substance produced by its action (or that of other *Cl. welchii* toxins) on muscle. They showed that though intravenous injection into rabbits of α-toxin in appropriate amounts leads to intravascular haemolysis, with haemoglobinaemia and haemoglobinuria, intramuscular injection of similar amounts of α-toxin has no such effect. They deduce from this that no serious amount of α-toxin enters the circulation after intramuscular injection, and that it cannot therefore be responsible for the coma, collapse and death that still occur. They support this further by showing that α-toxin cannot be recovered from muscle into which it has been injected one hour before the death of the animal, though it can readily be recovered from muscle injected after removal from the animal or from muscle injected in situ after death of its owner: nor can any α-toxin be obtained from human gangrenous muscle (MacLennan & Macfarlane, 1945). They suggest that this means that α-toxin is firmly adsorbed by the muscle of living animals, and that tissue fluids are no more likely to extract it from muscle than experimental procedures are; in which case it is unlikely that any α-toxin would reach the circulation. These arguments cannot be regarded as conclusive, as it is well known that the effect of even intravenous injection of some toxins (e.g., *Cl. septicum* filtrates (Lautenschläger, 1919)) depends on the speed with which they enter the circulation; a single dose injected intravenously at a very rapid rate is far more likely to kill than a larger dose divided up and injected over a long period. It seems reasonable to suppose that α-toxin injected intramuscularly would be absorbed very slowly, especially as it would be adsorbed on to the lecithin of damaged muscle, but that small amounts absorbed, even though they failed to produce haemolysis, might still produce serious changes in vital organs. Though α-toxin produces marked demyelination of brain slices, as would be expected from its lecithinase activity (Morrison & Zamecnik, 1950), it has no effect on the central nervous system after intravenous injection into living animals, as it does not pass the blood-brain barrier.

It is much more difficult to deal with the observations made by MacLennan & Macfarlane (1945) that, in patients suffering from gas gangrene who have received antitoxin, both normal and gangrenous muscle contain significant amounts of antitoxin, while little antitoxin can be recovered from the normal or the affected muscle of patients suffering from wounds unaffected by gas gangrene. Apparently the mere presence of antitoxin in muscle does not prevent the development of toxaemia and anaemia; it seems reasonable enough therefore to suppose that something not neutralized by antitoxin is responsible for the general changes in the patient. A fair comment is, however, that leakage of antitoxin may be very localized in wounds, and may not occur until irreversible damage has been done, though this does not explain why the bacteria continue to colonize antitoxin-containing muscle. Macfarlane & MacLennan (1945) drew attention to the great increase in extractable fat in gangrenous muscle, and to the possible production of lethal material by collagenase activity. Macfarlane (1946) also drew attention to the loss of adenyl compounds, creatine and potassium from damaged muscle as possible points of importance.

In an extensive series of experiments on the production of anoxia in the muscles of dogs, Kety, Nathanson, Nutt, Pope, Zamecnik, Aub & Brues (1945) had shown that a shock-like state often follows clamping of the triceps surae for five hours.

Removal of the ligature is followed by swelling of the injured limb. The fluid obtained by drainage of such damaged muscle has a pH of 7–7.2, and contains a little protein, probably derived from the circulation, and some haemolysed blood. Its phosphate and potassium content are increased (no doubt at the expense of the damaged muscle), its calcium content is low, and its bicarbonate content markedly reduced (Zamecnik *et al.* 1945). Injection of this fluid produced marked shock in about one-quarter of the dogs injected, but, as the effect was inconstant and the effusions contained bacteria, Aub, Brues, Kety, Nathanson, Nutt, Pope & Zamecnik (1945) ascribed the effect to a bacterial toxin. Pope, Zamecnik, Aub, Brues, Dubos, Nathanson & Nutt (1945) showed that the organisms were mainly *Cl. welchii* and staphylococci, that they increased enormously in number over the period of ligation, and that injection of *Cl. welchii* into anoxic muscles markedly increased the toxicity of the fluid that drained from them. Polyvalent gas gangrene antitoxin neutralized whatever substance was responsible for toxicity. There is no proof in these experiments that the toxic substance was any of the known toxins of *Cl. welchii*, but at any rate some antigenic toxic substance was present in anoxic muscle infected with *Cl. welchii*; it seems a pity that the experiments were not continued further until gas gangrene developed, and that the toxic substance was not identified. It is also worth noting that McClean, Rogers & Williams (1943) had no difficulty in demonstrating *Cl. welchii* α-toxin and hyaluronidase in the exudate in guinea-pigs infected with *Cl. welchii*, so that both their experiments and those of Pope *et al.* (1945) are in striking contrast to the findings of MacLennan & Macfarlane (1945). *Cl. septicum* produces a lethal toxin, a hyaluronidase and a deoxyribonuclease (Warrack, Bidwell & Oakley, 1951). The hyaluronidase and the deoxyribonuclease probably have much the same effect as those of *Cl. welchii*; the nature of the action of the lethal toxin, which is probably haemolytic, is unknown (Bernheimer, 1944). *Cl. oedematiens* type A produces a lecithinase (γ-toxin), a lipase (ε-toxin), an oxygen-labile lysin (δ-toxin), and a necrotizing lethal toxin (α-toxin), the latter being probably a vigorous capillary poison that increases the passage of protein and fluid from capillaries (Oakley *et al.* 1947; Menk, 1932). *Cl. oedematiens* type B has only once been recorded from a case of gas gangrene; it produces a lecithinase (β-toxin) distinct from that produced by type A, a haemolysin (ζ-toxin), and large amounts of α-toxin; it also produces a hyaluronidase, which has not been much investigated (Oakley *et al.* 1947; Keppie, 1944).

Apart from the capacity of *Cl. welchii* to attack collagen and gelatin, neither *Cl. welchii* nor *Cl. oedematiens* is notably proteolytic. *Cl. histolyticum*, however, besides producing a lethal toxin (α) of unknown nature, and a collagenase (β), produces at least two proteinases, a cysteine-activated proteinase (γ) and a cysteine-inactivated proteinase, not yet thoroughly investigated (Weil & Kocholaty, 1937; Maschmann, 1938; van Heyningen, 1940; Oakley & Warrack, 1950 ; Bowen, 1952).

5. Prophylaxis and Treatment

If we consider the state that has developed in a muscle affected with gas gangrene, in which vascular damage, haemorrhage, thrombosis and oedematous swelling have all reduced the entry of circulating blood, it may seem surprising that antitoxin therapy or chemotherapy should ever have been supposed to have any curative effect, except in very early cases, though they may unquestionably be of value in prophylaxis. It is exceedingly difficult to disentangle the effect of either form of treatment from the effect of surgery in human cases ; Macfarlane (1945) has claimed that antitoxin therapy is of value, since the incidence of gas gangrene and its mortality are lower in patients who have received antitoxin soon after wounding. Though animal experiments (Gordon & McLeod, 1941) suggest strongly that antitoxin in relatively small amounts has a marked prophylactic effect, and it is the general view that antitoxin is a useful adjunct to surgery and chemotherapy (MacLennan, 1943c), it is doubtful whether M. G. Macfarlane's evidence is very convincing, as the comparison between those who receive antitoxin late and those who receive it soon after wounding is not limited to this difference, since persons who have received it late will often have suffered from exposure on the battlefield for considerable periods, with corresponding adverse effects on their chances of recovery. MacLennan (1943c) was convinced that antitoxin was of value in treatment, though he admits that the number of cases he had did not justify more than an opinion; Jeffrey & Thomson (1944) were doubtful of the value of antitoxin, and state that its use does not affect the death-rate; Langley & Winkelstein (1945) thought that it had some postoperative, but no pre-operative value. North (1947) considers that it is of no value. Stock (1947) found mixtures of *Cl. septicum* and *Cl. welchii* antitoxin of little prophylactic value, but thought that this was due to the presence of *Cl. oedematiens* in the wounds.

There is a considerable literature on the chemotherapy of experimental gas gangrene (Gordon & McLeod (1941), sulphonamides; McIntosh & Selbie (1943a, 1943b), sulphonamides, flavines; Hac (1944), penicillin; Siebenmann & Plummer (1945), marfanil; Altemeier, Furste & Culbertson (1947a), penicillin, streptomycin; Sandusky & Keeble (1949), bactracin; Altemeier, McMurrin & Alt (1950) and Sandusky, Keeble, Wharton & Taylor (1950), chloromycetin, aureomycin; Taylor & Novak (1951), procaine-penicillin). The general conclusions seem clear: the best effects are obtained by prophylactic use of chemotherapeutic agents, particularly by local application; a definite reduction in mortality may be expected from any effective drug, though the dose necessary may be very high, up to about six hours after infection of damaged muscle; after this time no drug has any effect on the mortality. Intravenous or oral treatment is of less value than local treatment; mixtures of drugs, or of drugs and antitoxin, seem of greater value than any of them singly. It seems clear that success or failure of local or parenteral chemotherapy is likely to be determined in the first few hours after injury, in the short time during which the changes in the anoxic muscle are preparing the ground for the growth of anaerobes; after that, unless the damaged muscle is excised, chemotherapy is unlikely to be effective.

As might be expected, the value of chemotherapy in treatment is far less certain. MacLennan (1943d) had no evidence either way; Macfarlane (1945) claimed that penicillin had no useful effect on the mortality rate in gas gangrene cases that she reviewed, but there is some evidence (Altemeier *et al.* 1947a) that the amounts of penicillin used in treatment were seldom adequate; they recommend a total of 8 million i.u. per day. Cutler & Sandusky (1944) thought penicillin of little use without surgery; Jeffrey & Thomson (1944) and Langley & Winkelstein (1945) thought penicillin of value only

if radical surgery had been used; Birchall (1948) found that neither penicillin nor sulphadiazine was of any value. Stock (1947) thought even prophylactic sulphonamides of little value, and Langley & Winkelstein (1945) found them useless in treatment, either local or systemic.

One form of treatment that seems to have attracted little attention is that of Hinton (1947), who injected large volumes of oxygen through numerous punctures into skin and muscle just beyond the advancing edge of the gangrenous lesion developing after compound fracture of the radius and ulna. He claims a good result in one case, though as extensive surgery had been carried out, and penicillin, sulphamezathine and antitoxin had all been given, the effect of oxygen injection is not easy to evaluate. It is of interest that Hinton found that oxygen was absorbed more readily from diseased than from healthy tissue; it seems unlikely that injection of oxygen would produce much change in E_h unless it were well mixed and chemically combined with the necrotic muscle; diffusion would hardly produce much effect.

Almost all accounts of gas gangrene in man insist on the importance of radical surgery. Thus Jeffrey & Thomson (1944) considered radical surgery essential, and concluded that the anatomical situation of wounds exerts its effect not by providing more or less easy access to contamination, but by determining the extent to which excision is practicable. They consider, for instance, that wounds of the buttock, which usually allow of reasonably easy excision, are far less fatal than wounds of the thigh, where radical excision is far more difficult. If only partial excision is practicable, penicillin may be of value in treatment. Their views on mortality and site of wounding are supported by the figures of MacLennan (1943c). MacLennan (1946) has emphasized that therapeutic antitoxin will have little or no effect if any necrotic tissue is left, and has suggested that this is evidence against the role of *Cl. welchii* α-toxin in toxaemia; North (1947) considers radical surgery essential, and has little use for other measures; Langley & Winkelstein (1945) consider penicillin of great value after radical surgery.

It is not perhaps surprising that efficient and extensive surgery should be effective in the treatment of gas gangrene. If all damaged muscle is removed, the great bulk of infecting organisms will be removed with it, and the E_h in the adequately vascularized muscle will be far too high to allow the few remaining organisms to grow in it. This view is supported by the interesting experiments of Evans (1947), who showed that in experimental *Cl. welchii* gas gangrene in guinea-pigs, antitoxin or penicillin would save infected animals, but that the greatest curative effect was produced by incising the gangrenous muscle along its length. By this means, presumably, the tension in the muscle was reduced, the compressed capillaries reopened, and as the muscle became revascularized the E_h of the tissues rose above the point at which *Cl. welchii* could grow. These experiments also illustrate a further difference between gas gangrene in the guinea-pig and in man; in the guinea-pig the local damage produced by injection of calcium chloride is limited and unaccompanied by gross vascular change, so that once the increased pressure due to the swelling of the damaged muscle has been released, revascularization occurs readily. In man local damage is usually severe and in the majority of cases it is associated with severe and irreversible interference with the vascularization of the muscle.

Not only must surgery be adequate, but it must also be applied as early as possible. The longer anoxia persists in muscle, the more the pH will fall; and the greater the consequent degree of proteolysis, the higher the E_h at which pathogenic anaerobes will grow.

Earlier work on immunization against gas gangrene with formol toxoids has been summarized by Oakley (1943). In general the results were poor, though if large amounts of toxoid were injected into guinea-pigs, protection against 1,000 minimum lethal doses of *Cl. welchii* might be obtained. Injection of *Cl. septicum* toxoids gave fair protection against *Cl. septicum*; *Cl. oedematiens* toxoids gave no protection against *Cl. oedematiens* (Kolmer, 1942).

More recently Bernheimer (1947) has shown that injection of various preparations of *Cl. septicum* toxoid into man will induce the production of antibody against the lethal toxin of *Cl. septicum*; similarly, injection of a tervalent toxoid (*Cl. welchii*, *Cl. septicum*, *Cl. oedematiens*) induced the production of antibody to the lethal toxins of all three anaerobes.

Tytell, Logan, Tytell & Tepper (1947) showed that *Cl. welchii* and *Cl. oedematiens* toxoids induced good antibody production in mice, guinea-pigs, rabbits, goats, dogs and man, and gave good protection in animals. Altemeier, Furste, Culbertson, Wadsworth, Tytell, Logan & Tytell (1947b) found that their *Cl. welchii* toxoid, which in their first preparations protected only about 20% of guinea-pigs against infection, could be improved to give 50–100% protection. Interestingly enough, it was much easier to protect guinea-pigs against infection of healthy muscle than against infection of damaged muscle. Altemeier, Coith, Sherman, Logan & Tytell (1952) showed that guinea-pigs could be protected against *Cl. welchii*, *Cl. oedematiens* and *Cl. histolyticum* by injection of the appropriate toxoids; intramuscular injection of toxoid gave better results than subcutaneous injection. Purified *Cl. histolyticum* collagenase toxoid, plain or alum-precipitated, appeared to give better protection against *Cl. histolyticum* than did *Cl. histolyticum* filtrate, plain or alum-precipitated. No details are given of the purity of the *Cl. histolyticum* preparations, and the difference in survival rate is small, and may not be significant. Glenny's extensive experiments on the immunization of man remain unpublished. None of the preparations has yet been put to practical use or test in the field.

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TETANUS

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1 Pathogenesis of tetanus
2 Tetanus toxin
3 Site of action of tetanus toxin
4 Spread of the toxin in the body
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Although tetanus has usually been regarded as typically a complication of war injuries, the mortality from this disease in civil life is not negligible. In England and Wales in 1951, the Registrar-General recorded 81 deaths from tetanus, a number which may be compared with 33 from diphtheria—a disease which it resembles in being virtually preventable by simple prophylactic inoculation. In some European countries —for Italian statistics, see D'Antona (1952)—the death-rate may be many times higher. In Great Britain, the mortalities, when distributed by sex and age, show two clear maxima, the first during childhood and the second in later middle life (see fig. 1). While the low mortality of adolescents and young adults is probably partly attributable to their greater resistance to such complications of tetanus as pneumonia (Cole, 1940), it has almost certainly also been affected by the widespread active immunization with tetanus toxoid of young men and women in the armed forces. This inference is supported by the finding that when the mortalities at different ages for 1948–51 are expressed as percentages of those for 1928–37, the improvement has been conspicuously greater in persons in the age-groups 15 to 24 years, and 25 to 34 years, than in those either older or younger (see fig. 2). Indeed, in the age-group 1 to 5 years, the mortality in the last few years has actually been higher than it was 20 years before.

FIG. 1. MEAN ANNUAL MORTALITIES FROM TETANUS, BY SEXES AND AGES, IN ENGLAND AND WALES, DURING THE PERIOD 1948 TO 1951

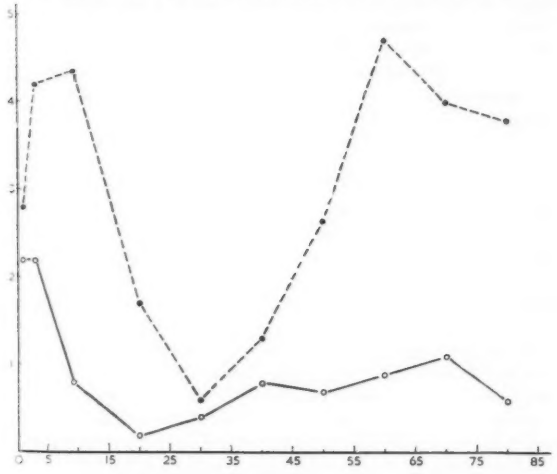
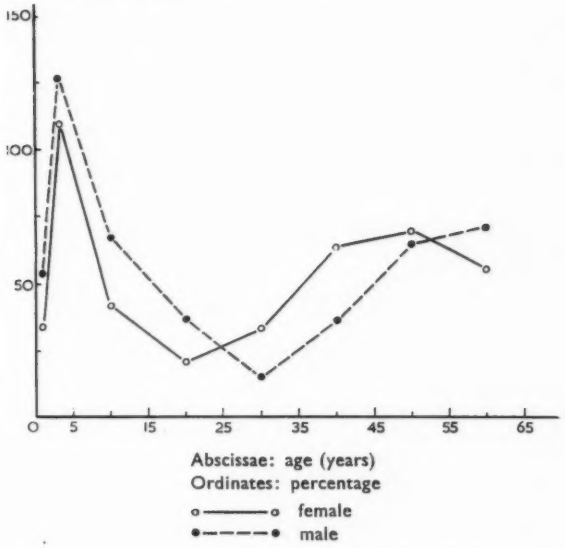


FIG. 2. MEAN ANNUAL MORTALITIES FROM TETANUS, BY SEXES AND AGES, IN ENGLAND AND WALES, DURING THE PERIOD 1948 TO 1951, EXPRESSED AS PERCENTAGES OF THOSE FOR 1928 TO 1937



In England and Wales (where deaths are referred to the registration area of the person's domicile), mortalities from tetanus are now notably higher in rural than in urban districts (Conybeare & Logan, 1951), an epidemiological distribution which is probably associated with the greater risk in agricultural communities that injuries may be contaminated with clostridial spores. How slight the initiating trauma may often be is shown by observations of Cole & Spooner (1935) on a series of cases collected from the much-farmed countryside round Cambridge. But although emphasis is rightly laid on the frequency of tetanus amongst agricultural workers, it should not be overlooked that in spite of the great improvement in neonatal care its incidence in early infancy still continues to be high.

1. Pathogenesis of Tetanus

The studies made by Fildes and his collaborators (Fildes, 1927, 1929a, 1929b; Knight & Fildes, 1930), in which they followed up earlier work by Bullock & Cramer (1919) and Tulloch (1919), have thrown much light on the circumstances under which clinical tetanus may supervene on an injury in which the damaged tissues have been contaminated with tetanus spores. In a series of experiments, made both in

vivo and in vitro, they showed that the power of the spore to germinate is closely dependent upon the oxidation-reduction potential (E_h) of the tissue or culture medium that immediately surrounds it. If, in consequence of local necrosis or of ischaemia from arterial ligation (Fildes, 1927), or even as a result of general anoxia from breathing mixtures low in oxygen content (Campbell & Fildes, 1931), the oxidation-reduction potential becomes lowered, the contaminating spores evolve into their vegetative form and multiply rapidly at the site of infection.[1] In cultures of the spores maintained within physiological ranges of pH, but poised at varying E_h potentials, Knight & Fildes (1930) showed that germination fails to occur when the E_h exceeds 0.11 v, but takes place within a few hours when the potential falls below zero. Once the vegetative form has begun to multiply, the toxin is formed rapidly; Francis (1914) recovered more than a thousand lethal doses by extraction of the tissues surrounding the site of inoculation of spores in experimental infections of guinea-pigs. On the other hand, when the oxidation-reduction potential exceeds the critical value, the spores persist, and although many may be ingested and destroyed by immigrant leucocytes, some may long continue latent in the tissues (Russell, 1927) and remain capable of germination, perhaps months later, should more favourable conditions develop. Such persisting spores are responsible for the occurrence of clinical tetanus in certain patients whose originally contaminated area of injured tissues has been re-explored surgically months afterwards without adequate prophylactic precautions (Weber, 1944).

2. Tetanus Toxin

Modern methods of purifying proteins have been applied by Pillemer and his collaborators to the separation of tetanospasmin from the crude toxic filtrates from cultures of toxigenic organisms (Pillemer & Robbins, 1949). Using cold methanol in buffered solutions of specified pH and ionic strength, they have prepared a toxin that is lethal to mice in doses of 0.09×10^{-2} μg., a potency much in excess of that of most earlier concentrates (Table I). This refined toxin, which has a high degree of purity as shown by its crystallizability, is, however, rather unstable, and tends to become converted even at low temperatures into a highly antigenic toxoid. Its use in experimental studies on the mode of action of tetanospasmin is consequently limited, though in experimental intoxications of mice Wartman & Pillemer (1949) found that the symptomatology and mode of death after the injection of such purified preparations did not differ recognizably from those with the more classical, though less potent, ammonium sulphate-precipitated toxins. In its most purified form, tetanus toxin is a protein with a minimum molecular weight of about 67,000 (Pillemer, Wittler, Burrell & Grossberg, 1948). It has an elemental constitution characteristic of most proteins, but, like botulinum toxin, it is distinguished by a notably high content of aspartic acid.

TABLE I. PURIFICATION OF TETANUS TOXIN

Preparation of toxin	Precipitant employed	Mouse m.l.d. (approx.) (μg. × 10^{-3})
Llewellyn Smith (1942–43)	Ammonium sulphate	1,500
Wright, Morgan & Payling Wright (1950)	Ammonium sulphate	30
Eaton & Gronau (1938)	Cadmium chloride	12
Pickett, Hoeprich & Germain (1945)	Cadmium chloride and ammonium sulphate	0.14
Pillemer, Wittler, Burrell & Grossberg (1948)	Cold methanol	0.09

[1] See Oakley, p. 52 of this number of the Bulletin.—Ed.

In recent years, with more careful titrations of potencies, evidence has accumulated that the ratio of the minimum lethal dose of tetanus toxin for various common laboratory animal species may differ significantly from one filtrate preparation to another. In a comparative assay of five preparations, Llewellyn Smith (1942–43) found that this ratio for rabbit and guinea-pig varied between extremes of 354 and 2.9, while that for mouse and guinea-pig ranged only between 6.25 and 2.8. From these and similar earlier observations, the inference was drawn that the culture filtrates from which these five toxin preparations had been derived contained two or more effective lethal constituents which differed relatively both in their distinctive toxicities for the various species and in the proportions in which they were present in the material injected. Whether or not this belief is correct, however, there are no indications from toxicological and immunological experiments that the various hypothetical toxic constituents differ as regards either their mode of action on the nervous system or their antigenic specificity.

3. Site of Action of Tetanus Toxin

Whilst it has been generally agreed that local tetanus can develop only in limbs in which the proprioceptive reflex mechanisms are intact, there has been no unanimity as to the point or points in this reflex pathway at which the toxin acts. Three possible sites for its action, each with its proponents, require consideration: (i) the myoneural junction; (ii) the proprioceptive sensory nerve-ending; and (iii) the central synapse. Except for Pochhammer (1909), who believed that the toxin brought about focal alterations in the myelin sheaths of both the afferent and efferent fibres in the motor nerve trunk—the alterations leading to a "short-circuiting" of proprioceptive reflexes at such sites—no one has suggested that the nerve fibres themselves are affected along their course.

i. *Myoneural junction.* Harvey (1939) undertook an examination of the myoneural junction in local tetanus in the limb muscles of cats. Using both belly-to-tendon leads and Adrian-Bronk concentric needle electrodes, he found that, after the motor nerve to the tibialis anticus muscle had been divided, a single nerve volley applied to the peripheral stump gave rise to repetitive muscle action potentials, a recording which contrasted strongly with the sharply-cut single response found with the normal uninoculated muscle. From this he concluded that the toxin had brought about some change at the myoneural junction which had the effect of converting a single excitation into a brief tetanus, and he thought that this alteration might be attributable to an abnormal readiness with which acetylcholine became liberated from its depot in the motor nerve-endings.

Such observations naturally attracted attention, and soon led to several further examinations of the problem with modern neurophysiological equipment. So far, no one has been able to confirm Harvey's main finding—the repetitive nature of the

muscle response in local tetanus to single impulses reaching the muscles through their motor nerve. Göpfort & Schaefer (1940) could find no evidence of any such after-discharge at the myoneural junctions, and suggested that Harvey's findings might be accounted for by the spread of electrotonus from the stimulating electrodes on the nerve trunk to the nearby muscle end-plates themselves. Acheson, Ratnoff & Schoenbach (1942) and Perdrup (1946) similarly found no repetitive action potentials to single stimuli applied to the cut nerves of inoculated muscles. The consensus of belief thus runs counter to the belief that the myoneural junction is affected by tetanus in such a way as to replicate the excitation at the nerve-ending and thus maintain spasticity in the regional muscles.

ii. *Proprioceptive sensory nerve-endings.* Since Sherrington (1906a) first drew attention to the similarities between tetanus and decerebrate rigidity, it has been widely supposed that the muscular spasticity evoked by the intoxication was associated with an exaggeration of proprioceptive reflexes. This belief was supported by the studies of Liljestrand & Magnus (1919) on postural control and by those of Ranson (1928) on muscle tonus; in both studies, tetanus toxin was used to aid in elucidating the mechanism of proprioceptive activity. From these investigations, it became apparent that the overactivity of the muscles in local tetanus could arise either from a pathological sensitivity of the peripheral receptors to tension or from some modification of transmission at the central synapses. The chief support for the former view has come in recent years from Schaefer (1944) and Perdrup (1946).

Schaefer's belief was founded mainly on two observations: firstly, that the action potentials recordable after a quick stretch from the distal part of the divided motor nerve trunk were markedly augmented if the related muscles had previously been injected with toxin; and secondly, that the electromyograms obtainable after crossed stretch reflexes in control and in intoxicated limbs showed greater frequency and amplitude when the tension was applied to the tetanus side and the recordings made from the control one than vice versa. Neither observation is conclusive. The former is explicable by the physical change in the elastic properties of muscle that has been continuously in spasm for several days and by the effect that this contracture has upon the stimulation of the local proprioceptive sense organs by the stretch. The latter is more reasonably attributable to a regional intoxication of the spinal cord that falls more heavily on the synapses on the inoculated side.

Perdrup based his conclusions solely on the observation that an injection of a dilute solution of procaine into a spastic muscle in local tetanus abolished its characteristic electromyogram without affecting its normal voluntary or reflex activity. This finding, too, is inconclusive, for it fails to distinguish between an augmentation of the proprioceptive reflex activity of the affected muscles that depends on an increase in the number of centripetal impulses originating in the supposedly intoxicated sensory nerve-endings, and one in which the number of such afferent impulses is normal but becomes multiplied through after-discharge at diseased spinal cord synapses.

iii. *Spinal cord synapses.* An observation made by Meyer & Ransom (1903) and by Sherrington (1906b), and confirmed by many since, which seems impossible to reconcile with any theory that the intoxication lies peripherally either in the muscle end-plates or in the proprioceptive sensory nerve-endings, is that the local tetanus brought about by intraneural injections of the toxin high in the regional motor nerve trunks to a limb is indistinguishable from that which develops after an inoculation into the regional muscles themselves. This conclusion is materially strengthened by the finding (Baylis, Mackintosh, Morgan & Payling Wright, 1952) that, if the toxin was injected into the calf muscles of a rabbit whose sciatic nerve had previously been sclerosed by an intraneural injection of a small quantity of a mildly irritant solution in the mid-femoral region, no local spasticity developed. Yet with this intramuscular route of injection, both the motor and the sensory nerve-endings in the muscles were exposed to an inoculum of the toxin that produced typical local tetanus in animals used as controls. To exclude the possibility that the nerve sclerosis might in some way have impaired the ability of the muscles to react to the toxin, a small amount of it was subsequently injected into the nerve trunk proximal to the site of sclerosis; typical local tetanus promptly followed.

Many observations are in harmony with the view that local tetanus is a manifestation of a regional central nervous system intoxication (see Wright, Morgan & Payling Wright, 1952). It has been repeatedly noted that when an intramuscular or intraneural inoculum of the toxin exceeds substantially the minimum needed to evoke spasticity in the injected limb, a similar though less severe degree of rigidity may appear soon afterwards in the limb muscles on the opposite side. Moreover, when the toxin is injected intraneurally into the vagus, facial or hypoglossal nerves of rabbits (Wright, Morgan & Payling Wright, 1950), disturbances indicative of central intoxication, such as salivation, strabismus, torticollis and bradycardia, become apparent in quick succession after a characteristic latent period. All the effector structures involved in producing these signs are widely separated anatomically, yet all receive their motor innervation from nuclei that are in close propinquity in the brain stem. Finally, local tetanus in a limb, following a distal intramuscular inoculum of toxin, can be wholly averted by the injection of a small amount of antitoxin into the regional spinal cord either at the same time or within a few hours subsequently (Permin, 1914).

4. Spread of the Toxin in the Body

The search for the route taken by the toxin from its site of formation within the tissues (or its site of introduction into them) to its destination in the central nervous system has given rise to an extensive and controversial literature. The essentially nervous character of the clinical manifestations, and their limitation in local tetanus to the muscles near the site of infection, early suggested the possibility that the toxin spreads to the spinal cord along the regional motor nerve trunks. The emergence of this concept of hodogenesis from its early nebulous origins into the form of a well-sustained hypothesis was largely the result of a penetrating analysis published by Meyer & Ransom (1903) just half a century ago. It was these authors who chose and strongly fortified the position on which so many ineffectual assaults have since been made.

Briefly, Meyer and Ransom based their contention that tetanus toxin injected peripherally into a limb reaches the central nervous system by way of nerve trunks on the following observations, all of which have since been repeatedly confirmed: (i) after such an injection, the toxin can be recovered from the regional nerve trunks; (ii) the minimum lethal dose

for cat, dog and rabbit is significantly less when the toxin is injected into a large mixed nerve trunk than when given subcutaneously; (iii) an injection of specific antitoxin into a large trunk, such as the sciatic nerve, averts local tetanus when the toxin is injected distally in the same limb; (iv) typical tetanus develops in the region innervated when toxin is injected directly into the spinal cord.

The most persistent criticism of the view that tetanus toxin spreads centripetally, initially along motor nerve trunks and later up the spinal cord, has come from Abel and his collaborators in Baltimore (Abel, Evans, Hampil & Lee, 1935; Abel & Hampil, 1935; Abel, Hampil & Jonas, 1935; Abel, Firor & Chalian, 1938). Their main grounds for dissenting were firstly their failure to prevent the development of local tetanus by the proximal intraneural injection of antitoxin and secondly their inability to produce a condition that they could accept as local tetanus by the intraneural inoculation of toxin. In weighing the value of this latter contention—which is at sharp variance with results described by many others who have made the same experiment—it would seem to be not without significance that more than half the dogs they so inoculated later developed "stiffness" in the injected leg. Neither of Abel's objections seems valid; reasons for rejecting them both have been given elsewhere (Payling Wright, 1953). To those there set out, may here be added two further material pieces of evidence against the Baltimore view that tetanus toxin spreads from its depot in the tissue to the central nervous system exclusively by the blood stream.

D'Antona (1949, 1951) has recorded an experiment in which the administration of toxin and antitoxin to guinea-pigs was subjected to various combinations of route and amount. His main findings are condensed in Table II. From this table, it is apparent that intravenous doses of antitoxin which are capable of preventing fatal tetanus when the toxin is given directly into the blood stream are incapable of doing so when the injection is made intramuscularly into a limb. Yet this latter mode of administering the toxin fails to kill the animals if the regional motor nerves to the injected muscles have previously been resected.

TABLE II. EFFECTS OF ROUTE OF INJECTION OF TETANUS TOXIN

Toxin		Prior treatment	Intravenous antitoxin (i.u.)	Result
Dose (mg.)	Route			
17.5	Intravenous	None	25	Survived ; no tetanus
17.5	Intravenous	None	50	Survived ; no tetanus
17.5	Intramuscular	None	25	Death after 6 days
17.5	Intramuscular	None	50	Death after 10 days
17.5	Intramuscular	Nerve	25	Survived ; no tetanus
17.5	Intramuscular	resected	50	Survived ; no tetanus
7.5	Intravenous	None	None	Death after 2 days
7.5	Intravenous	None	None	Death after 2 days

The observations of Friedemann, Hollander & Tarlov (1941), to which they have applied the term "route phenomenon", are consistent with those of D'Antona, and complementary to them. They found that if 10 to 20 minimum lethal doses of toxin are injected into guinea-pigs, in one group intravenously and in the other intramuscularly, it requires from 20 to 80 times more antitoxin in the blood to protect the latter animals effectively. Their interpretation of this experiment is that the toxin introduced into the muscles gains access to the vulnerable nuclei in the central nervous system by some route along which it is sheltered from the neutralizing action of the circulating antitoxin. Like Meyer and Ransom, they believe this route to be the regional motor nerve trunks.

In fatal ascending tetanus, there are grounds for believing that the toxin moves in a cranial direction within the substance of the spinal cord, and that death results from intoxication of the bulbar nuclei. Many experiments have shown that, when comparative inoculations are made into the blood stream, limb muscles, large nerve trunks, spinal cord and medulla oblongata, the minimum lethal dose of tetanus toxin decreases progressively and steeply (Firor, Lamont & Shumacker, 1940; Wright, 1953). Indeed, the lethal inoculum into the medulla oblongata is only about one five-hundredth of that needed when the toxin is injected directly into the blood stream. The transportation headward of only a small fraction of the toxin that enters the lumbar spinal cord may therefore suffice to kill the animal. That such a movement can take place seems likely from experiments in which use is made of radioactive tracer proteins. When a small volume of saline containing tetanus toxin together with radioiodinated homologous serum proteins was injected into the sciatic nerve trunk, the rabbits died with ascending tetanus. Determinations of the radioactivity of samples taken at various levels of the spinal cord and medulla oblongata in these animals showed that at the time of death a significant fraction of the radioactive material had reached the brain stem (Wright, Morgan & Payling Wright, 1951). Lastly, it has been demonstrated by many investigators that the transection of the spinal cord between the site of inoculation and the brain stem averts a fatal intoxication (Meyer & Ransom, 1903; Firor *et al.* 1940; Friedemann *et al.* 1941; Baylis, Joseph, Mackintosh, Morgan & Payling Wright, 1952). It would seem, therefore, that in progressive ascending tetanus the major regional nerve trunks and the spinal cord form a continuous pathway along which the toxin spreads from its depot in the tissues to cause death by intoxication of the bulbar nuclei.

5. Modes of Action of Tetanus Toxin

Very little is known about the nature of the biochemical lesion produced in the neurones by tetanus toxin. From the very minute size of the minimum lethal dose and from the well-known effect of variations in temperature on the progress of the intoxication in poikilothermic animals (Courmont & Doyon, 1899; Cowles & Nelson, 1947), it seems possible that it operates as an enzyme, like the toxins of several other clostridia, and that its putative substrate is present exclusively in nerve cells. When injected into the anterior chamber of the rabbit's eye in sublethal doses, it produces a characteristic paralysis of the cholinergic sphincter pupillae of the iris (Ambache, Morgan & Payling Wright, 1948a). In thus injuring the cholinergic mechanism and sparing the adrenergic innervation, it resembles the comparably potent toxin of *Clostridium botulinum* Type A (Ambache, 1949). Though it might appear from these observations that both toxins in some way impair the liberation of acetylcholine (Ambache, Morgan & Payling Wright, 1948b; Burgen, Dickens & Zatman, 1949), the *modus operandi* is still unexplained and

the obscurity is further increased by the finding that whereas tetanus toxin is exceedingly lethal when injected into the medulla oblongata, botulinum toxin possesses no comparable action when inoculated directly into the central nervous system (Davies, Morgan, Wright & Payling Wright, 1953).

6. Active Prophylaxis against Tetanus

Experience with troops during the Second World War showed incontestably that the simple prophylactic measure of inoculation with toxoid can almost wholly eliminate tetanus under circumstances in which there is serious risk of contracting the disease (Boyd, 1946; Ramon, 1949; D'Antona, 1952). The value of the protection thus conferred became particularly evident during the military operations in western Europe in 1944 and 1945, for the incidence of tetanus amongst German wounded prisoners, who had not been actively immunized, was very much higher than amongst British wounded from the same battle areas. In his interpretation of these findings in the light of current views in immunology, Boyd points out that

> The mechanism of active-immunity may be regarded as functioning in two consecutive phases: first, neutralisation of toxin by preformed antitoxin (deriving from the last inoculation with tetanus toxoid) which is circulating in the blood-stream; and secondly, at a later stage, neutralisation of toxin by antitoxin newly fabricated as the result of the toxin stimulus to the previously sensitised reticulo-endothelial system.

Even were the antitoxin titre of the serum of the injured person at the time of infection with the spores well below a protective level, should he possess a previously experienced antitoxin-forming apparatus the stimulus of the toxin would suffice to evoke the liberation of a large amount of neutralizing antibody within a period much shorter than the usual incubation time of this disease. It is because of this time relationship that active immunization against tetanus and diphtheria is so much more feasible than against gas gangrene, the incubation period of which is usually much too short to permit any such "secondary response" that might effectively mitigate the course of the intoxication.

The success of active immunization of personnel of the armed forces has naturally directed attention to the desirability of introducing similar prophylactic measures in civil life. Although the disease is uncommon in Great Britain (about 200 cases yearly in England and Wales), its distressing clinical course and high case-fatality rate encourage every effort for its prevention, especially when this can be virtually accomplished by so simple a prophylactic procedure. As with diphtheria, French immunologists, headed by Ramon, have taken a leading place in developing practical methods for the active protection of human beings against tetanus; it is hardly surprising, therefore, that the first large-scale scheme for the active immunization of young children against this disease should have been devised in France (Ramon, 1950). In 1943, the general prophylactic inoculation of young children against both tetanus and diphtheria was made obligatory at Lyons, and the practice has since spread to other places in Europe and North America. Fortunately, it has been found practicable to combine within a single injection the antigens needed both for diphtheria and tetanus (Bigler & Werner, 1941; Bigler, 1951); sometimes pertussis vaccine (Miller & Ryan, 1948; Miller, Ryan & Beard, 1949; Fleming, Greenberg & Beith, 1948; di Sant'Agnese, 1949, 1950; Sauer & Tucker, 1950; Murray, 1950) and sometimes typhoid-paratyphoid vaccine (Maclean & Holt, 1940; Régamey & Grumbach, 1941) are also incorporated in the inoculum given. In estimating the practical considerations involved in such multiple prophylaxis, it is worthy of note that titrations of the heights to which the respective antibodies rise subsequently have shown that this combined immunization, far from detracting from the response to any of the constituent antigens, markedly favours the production of antibodies for each.

Since it is now well established that simultaneous prophylaxis with more than one antigen is not only feasible but possesses material advantages, there seems to be no valid reason why these combined immunizations should not be more widely employed as a public health measure in Great Britain. They need entail neither an additional nor a larger injection, for both the age at which it should be given and the time interval that separates the successive inoculations are the same as those at present current for diphtheria toxoid alone. To attempt specific immunization against tetanus either on a topographical or on an occupational basis, as recommended by Conybeare & Logan (1951) and by Boyd (1951) respectively, would unquestionably raise greater administrative problems and offer less reward than would a general application of combined prophylaxis with diphtheria and tetanus toxoids to all children in early life. Furthermore, such early immunization would be effective at the time of life when the mortality from tetanus is at its peak. It is to be hoped that more public health authorities, especially in countries where the death-rate from tetanus is high, will take advantage of the simple and inexpensive protective measures against this disease that are now unquestionably effective and readily available.

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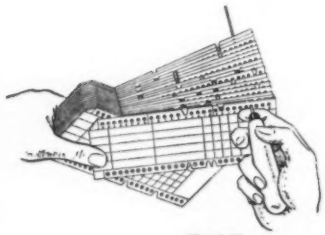

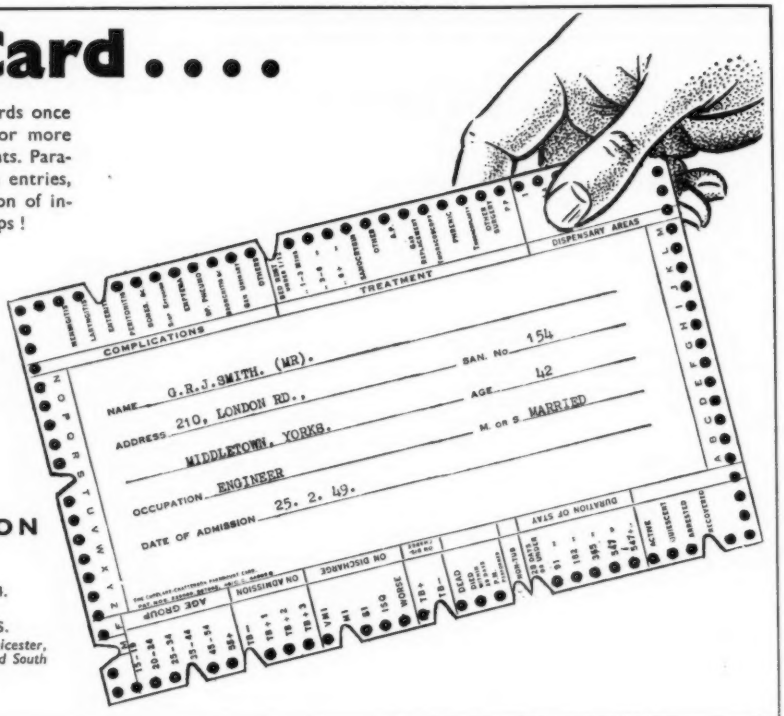

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STUDIES ON HEAD INJURY

W. RITCHIE RUSSELL C.B.E. M.D. F.R.C.P.

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1 Mechanism of concussion
2 Contrecoup injuries
3 After effects of head injury
4 Management of the post-concussional syndrome
5 Research on the effects of focal brain wound
6 Prevention of head injury
References

Injury to the brain, spinal cord or peripheral nerves may destroy neurones or block conduction in them and may tear blood vessels supplying the injured tissue, causing areas of haemorrhage or reactionary oedema. Injury to the skull or spine may cause displacement of bone or dislocation, which may not only destroy nervous tissue but may cause compression and local ischaemic necrosis.

Medical opinion is still divided as to the relative importance of these factors in certain forms of injury but there is now much more agreement regarding the mechanism of concussion than there was, say, twenty years ago.

1. Mechanism of Concussion

Although many ancient writers attributed the consequences of concussion to a shaking or commotion of the brain (see an interesting historical chapter in *Commotio cerebri* by Courville (1953)), yet many other factors have in turn been put forward during the past century to explain the clinical facts. In the first place the occurrence of capillary haemorrhages in the brain in certain fatal cases of head injury led to the view that these haemorrhages formed an essential factor in causing concussion. Then the idea developed that concussion depended on acute cerebral anaemia, the injury squashing the brain so that the arrested circulation led to loss of consciousness. Another more recent tendency has been to ascribe most of the dangers of head injury to cerebral oedema, with the result that treatment by intensive dehydration has been favoured in some clinics.

The term " concussion " is often used to indicate a minor degree of injury from which complete recovery occurs and for which there is no visible pathology. There is, however, often no means of establishing either of these criteria in any one case, and so much is this the case that the attempt to distinguish between cerebral concussion and contusion is often futile. The pathologist likes to attribute death to an area of visible bruising on the surface of the brain (cerebral contusion), whereas it is often obvious that the visible areas of bruising are quite unimportant. Scattered capillary haemorrhages are occasionally observed throughout the brain in fatal cases of head injury, but these are not usually the cause of death. They are better regarded as merely an indication of the widespread forces to which the brain is subjected. Such appearances may also be due to fat embolism. There are many grades of neuronal injury which block conduction without visible change through the microscope: it is, in fact, what Jefferson (1942) called the invisible contusion of brain-stem centres which causes death.

The idea that cerebral anaemia might be the cause of concussion has become quite untenable. The experimental production of cerebral anaemia has effects which are much slower to develop than the instantaneous paralysis of concussion, while the pattern of recovery is entirely different.

It is therefore now generally accepted that the instantaneous paralysis of head injury indicates a direct traumatic effect on cerebral neurones (Russell, 1932; Symonds, 1935). Williams & Denny-Brown (1941) have demonstrated the electrical silence of the cortex after experimental concussion. Holbourn (1943) has calculated the rotational forces which must distort the neurones, while Pudenz & Sheldon (1946) have provided remarkable confirmation of the distorting waves of force in the brain by their brilliant high-speed cinematographic records of the effects of head injury observed through a lucite calvarium—a transparent replacement of a large area of skull in a monkey.

The essential feature of most instances of head injury is the sudden change of velocity imparted to the skull and brain. The experiments of Denny-Brown & Russell (1941) correlated the degree of brain commotion with the acceleration forces to which the brain is subjected. Their " acceleration concussion " is the type which generally occurs in accidents: either the stationary head is struck by a fast-moving blunt object or, more commonly, the moving head is suddenly arrested by a stationary surface such as the ground. They found that a crushing injury with the head fixed and unable to change velocity causes little brain damage unless the skull is severely smashed. Clinical experience also (Russell & Schiller, 1949) confirms that in man, if crushing injuries are survived, a frequent feature is that, though the skull be fractured and cranial nerves torn, consciousness is often fully preserved. The victim may actually walk away from the accident with blood pouring from his ears owing to fractures of the base of the skull.

2. Contrecoup Injuries

The occurrence of contrecoup injuries has for long provided food for speculation. It was at one time thought that this injury opposite the site of impact might be caused by the brain rebounding against the opposite side of the skull. Such a view is not, however, acceptable to the physicist, while the appearance of the contrecoup lesion (Russell, 1932) is usually distinctive and indicates a vascular lesion probably due to the brain tearing itself from its meninges by its own momentum, and thus tearing pia-arachnoid vessels. Russell visualized a linear momentum effect, but Holbourn (1943) has shown that rotational forces are more important and indeed has shown how the temporal poles and inferior frontal lobes are specially liable to contrecoup lesions. This latter often results in permanent anosmia due to tearing of the olfactory filaments.

It is apparent therefore that apart from vascular lesions of varying importance, the essential feature of head injury is the occurrence of distorting forces within the brain which paralyse or destroy neurones. All degrees of this commotio cerebri occur. Instantaneous paralysis and loss of consciousness is a presenting feature common to these injuries, while the time required for recovery seems to depend on the severity of neuronal injury or dysfunction. The term " concussion ", as used to indicate a certain degree of injury, ceases therefore to

have much meaning, and it seems better simply to use the term " concussion " to indicate the phenomenon of sudden paralysis of the brain that occurs in response to such acceleration insults.

Those who have viewed Pudenz and Sheldon's remarkable film will remember the to-and-fro rotational movements which developed in all visible areas of brain during head injury. Such movements must obviously temporarily block or even disrupt many neuronal pathways in the brain. The sudden loss of consciousness may be a consequence of this almost universal disturbance throughout the brain, or it may be due to the neuronal disorder involving a particularly vital brain-stem centre. Either of these mechanisms seems to be likely, on theoretical grounds, to produce the general concussion in the sense just referred to. In this connexion it should be pointed out that brain-stem lesions do not necessarily cause prolonged loss of consciousness. There is a well-established mid-brain syndrome following closed head injury (Kremer, Russell & Smyth, 1947) and in one of the cases of these authors the post-traumatic amnesia (PTA) was of only six hours' duration.

When a peripheral nerve is injured by accident or experiment (Denny-Brown & Brenner, 1944) in such a way as to allow recovery without the need for regeneration—what Seddon (1942) calls neurapraxia—then the time taken for recovery varies from a few minutes to three or four weeks. Similarly the recovery from spinal cord contusion varies from a few minutes to many weeks. In the brain also, where regeneration is impossible, we may reasonably accept the view that there are innumerable degrees of neuronal injury which take varying periods to recover. It is presumably for this reason that, in the uncomplicated case of head injury, the length of the period of disturbed consciousness varies with every case and indeed provides a useful means of classifying head injuries. The duration of PTA is therefore much used for this purpose (Symonds & Russell, 1943; Symonds, 1949). Table I illustrates the value of the duration of PTA in estimating prognosis.

TABLE I. PROGNOSIS OF 237 ACUTE CASES OF HEAD INJURY IN SERVICE PERSONNEL, BASED ON FOLLOW-UP REPORT AFTER RETURN TO DUTY (Symonds & Russell, 1943)

Duration of post-traumatic amnesia	Total number of cases in group	Percentage of cases in each group showing a successful return to duty
Nil	28	89
Under 1 hour	75	89
1–24 hours	65	88
1–7 days	34	76
Over 7 days	35	40
	237	80

Studies of the pattern of mental recovery after head injury (Russell, 1932) and of the memory disorders following head injury (Russell & Nathan, 1946; Williams & Zangwill, 1952) indicate that various mental mechanisms recover at different intervals after the injury. These stages of mental recovery resemble closely the mental recovery after anaesthesia, but after head injury the stages of mental recovery pass from one to another in a space of minutes, hours, days or even weeks, according to the severity of the original neuronal trauma. The effects on memory are of special interest. Severe head injury undoubtedly destroys many memory " traces " and here there is interesting evidence to indicate that the most recently established memories are the most vulnerable, and, as in old age, remote memories remain, even though many of these are of no special importance to the individual. It seems that many memories are strengthened by the passage of time irrespective of their importance, and it is suggested that this remarkable strength of remote memories may be effected simply by the spontaneous neuronal activity of the brain discharging through memory circuits almost continually, without the occurrence necessarily ever reaching consciousness.

During recovery of consciousness, whether slow or rapid, the various aspects of mental activity recover in an order according to their complexity. In this connexion, recovery of the patient's full awareness of his environment, along with the ability to record events for subsequent recall, provides one of the last stages of recovery, and it is for this reason that the duration of PTA is such a useful indication of the severity of brain damage in the average case.

These studies have also been concerned with retrograde amnesia (RA), and it has become clear that RA lasting for at least a few moments is almost invariably present after recovery from concussion. RA seems to depend on concussion obliterating memories which are in process of being recorded on the brain. Occasionally (Russell, 1935, 1948) this process gets interrupted " half-way " so that after recovery the patient may have a " vision " of something (for example, a running horse) which caused the accident but which fails to reach the ordinary channels of memory.

Studies of RA are complicated by the fact that, as has been mentioned, recent memories are in any case specially vulnerable to severe concussion, and recently acquired knowledge or skills may be largely obliterated.

3. After Effects of Head Injury

What then are the harmful effects of head injury which may lead to some permanent loss of efficiency? These evidences of brain damage are more often apparent in cases with prolonged PTA. In addition, however, age plays a most important part and the minor degrees of traumatic dementia are quite common in those who are over 50 years of age when injured. Repeated relatively minor injuries also become harmful, as in the " punch-drunk " syndrome, which is clearly a degree of traumatic dementia, often combined with an unfortunate psychological reaction to the disability.

The histophysiological basis for these varying degrees of traumatic dementia is not clear. Rand and Courville, among others, demonstrated degenerative changes in nerve cells after fatal head injury (Courville, 1953). It must be admitted, however, that histological techniques fail to elucidate fully the histopathology of traumatic dementia. Study of the blood-brain barrier in these cases (Zetterholm, 1947) merits more attention than it has yet received.

In general it may be said that simple psychometric tests, and evidence from relations and acquaintances, give more reliable evidence than any other method yet available with regard to changes in personal behaviour and ability following head injury.

4. Management of the Post-Concussional Syndrome

War-time experience in Britain led to a complete revolution in the treatment of accidental head injury and rehabilitation

TABLE II. DURATION OF INCAPACITY FOR FULL WORK IN AN UNSELECTED GROUP OF 139 WORKING MEN AND WOMEN ADMITTED TO EDINBURGH ROYAL INFIRMARY

(The cases are arranged according to duration of post-traumatic amnesia (Russell, 1933–34))

Period off full work	Duration of post-traumatic amnesia				
	Under 1 hour	1–24 hours	1–3 days	Over 3 days	Total number of cases in group
Under 2 months	37	30	2	4	73
2–6 months .	11	8	3	15	37
6–18 months .	4	3	—	3	10
Over 18 months	9*	4	—	6	19
Total . .	61	45	5	28	139

* In seven of these nine cases there was a compensation problem outstanding.

after it. The traditional use of rest and quiet for long periods was largely discarded, and graduated physical exercises were begun as soon as the patient's condition allowed (Symonds, 1935, 1941; Cairns, 1942).

The common symptoms, such as vascular instability, headache, sensitivity to head movement or to eye strain, were treated not by the avoidance of aggravating factors, but by their regular practice—by stooping and by head movements, orthoptic exercises and a general programme of increasing activity of all kinds. Special centres were established for the rehabilitation of these patients, and the results proved to be gratifying not only from the point of view of early full recovery, but also from the point of view of the avoidance of superadded psychological disturbances.

Return to work too soon seems to do no real harm provided that the patient is not unduly disturbed psychologically by his initial failure. He can try again later, but in the meantime requires some common-sense supervision to protect him from harmful influences at home, and so forth. Residential rehabilitation centres are specially useful for getting the best results in personal adaptation after head injury.

The actual duration of disability after various degrees of head injury in both civilians and soldiers was found to be much shorter than was often thought, as is seen in Tables II and III (see studies by Russell, 1933–34, 1942; Guttmann, 1943; Symonds & Russell, 1943).

TABLE III. DURATION OF TREATMENT IN A SERIES OF ACUTE SERVICE CASES WITH A SATISFACTORY FOLLOW-UP AFTER RETURN TO DUTY (Symonds & Russell, 1943)

Duration of treatment, from injury to return to duty (successful on follow-up)	Duration of post-traumatic amnesia				
	Under 1 hour	1–24 hours	1–7 days	Over 7 days	Total number of cases in group
Under 2 weeks	8	—	—	—	8
2–4 weeks .	27	10	1	—	38
1–2 months .	26	23	15	1	65
2–3 months .	19	12	5	7	43
3–6 months .	2	5	3	3	13
Over 6 months	—	—	—	—	—
Total . .	82	50	24	11	167

5. Research on the Effects of Focal Brain Wound

Modern warfare causes many brain wounds, and a large proportion of those patients who survive have been wounded by small metal fragments travelling at a very high velocity. These fragments cut through the skull or glance off its surface. In either case there seems to be an explosive effect at the point of injury so that a shower of bone fragments penetrates the brain for a distance of some centimetres. These provide many examples of focal brain wound, and after débridement and healing they provide unique material for research on brain function (see *British Journal of Surgery*, 1947).

The main centre in Britain for brain wound cases during the Second World War was at the Military Hospital for Head Injuries, Oxford. The records of that hospital have been carefully preserved, and a social Advice Bureau has been formed to assist and advise those who have had a brain wound. This Bureau is not only an interesting experiment in welfare,

TABLE IV. INCIDENCE OF POST-TRAUMATIC EPILEPSY IN 820 CASES OF PENETRATING BRAIN WOUNDS (SECOND WORLD WAR) AT FIVE-YEAR FOLLOW-UP (Russell, 1951)

	Number of cases	Percentage of total
Cases with fits on follow-up .	356	43
Cases with no fits, followed up for the following periods after injury:		
Over 5 years . . .	403	
2–5 years	21	
1–2 years	21	
Less than 1 year. . .	19	
Total cases with no fits . .	464	57

but provides a link with the cases, thus facilitating a number of research studies that have been continued since the war. Each case is recorded on a specially designed "paramount" filing card (Russell, 1951) which makes it easy to collect and analyse each of many types of focal disorders.

One remarkable finding from this study is that in spite of the tremendous advances in surgical technique in the Second World War, as a result of which 85% of the brain wounds healed by first intention (Cairns, 1948), the incidence of post-traumatic epilepsy has not been reduced below the figure for the First World War (Russell & Whitty, 1952; and see Table IV of this paper). Indeed, the incidence of epilepsy in these cases is approaching 50%, as it did after the First World War (Baumm, 1930; Credner, 1930). This figure of 50% suggests that a penetrating brain wound upsets the anatomical balance between two equal and opposing factors, which may presumably be simply the competing forces of excitation and inhibition. It is of great interest that the incidence of epilepsy following cured cerebral abscess is also about 50% (Jooma, Pennybacker & Tutton, 1951). The implications of these figures in regard to the application of neurosurgical techniques to the treatment of traumatic epilepsy have not yet been fully explored, but it is interesting to observe the tendency among

neurosurgeons to pay more attention to removing electrically unstable cortex near a brain scar, and less attention to removing the scar itself (Walker, 1949).

It should, of course, be explained that the occurrence of a single epileptic fit qualified the subject for a label of post-traumatic epilepsy. Functional recovery after brain wound is usually surprisingly good; and over 80% of those who suffered brain wounds in the Second World War were gainfully employed in 1950 (Russell, 1951).

During and after the First World War neurologists in many countries studied the effects of focal brain wounds, and in Britain the studies by Head, Holmes and Riddoch are well known. The same process has developed with regard to cases from the Second World War, and many such studies which have been made in Britain are now appearing in the *Journal of Neurology, Neurosurgery and Psychiatry*, and in *Brain*. For example, Marshall (1951) has made a fresh study of sensory disturbances in relation to cortical wounds, with special reference to pain sensibility, and has questioned some of the conclusions of Head & Holmes (1911). He found convincing evidence that, in some cases of cortical lesion, pain sensibility is greatly reduced, and this was particularly well demonstrated by the use of Kellgren's method of testing deep pain (Kellgren, 1938). This consists of the injection of 0.2 ml. of a 6% (w/v) sodium chloride solution into muscles.

Spalding (1952) has studied wounds of optic radiation and calcarine cortex, and has been able to throw light on the anatomy of the common quadrantic defects in wounds of the anterior optic radiation. Humphrey & Zangwill (1952) studied traumatic aphasia in left-handed patients, and have collected evidence to show that left-handed persons do not have a dominant hemisphere in the same way as do the right-handed. They seem to have a different organization of their speech mechanism. Russell & Whitty (1953), in one of a series of studies on post-traumatic epilepsy, analysed 85 cases with focal motor or somatic sensory attacks. Special attention has been paid to the direction of spread from one type of aura to another. For example, a sensory aura often spreads to a focal motor aura, but the reverse process is rare. Rare cases in which there is an aura of focal heat, cold, pain, agnosia or phantom movements get special attention. The possibility that the discharging cortex in a focal attack is, in fact, partially cut off from its afferents, is discussed, so the possibility that traumatic epilepsy is a release phenomenon cannot be excluded.

Most forms of focal (Bravais-Jacksonian) epilepsy may occur in either a positive or a negative form. On the motor side there may be spasms or paralysis of one limb, while in the sensory sphere there may be painful paraesthesias or numbness; flashes of light or hemianopia; a phantom sensation of one limb moving, or a sensation of one limb disappearing; an agnosia. The detailed study of these phenomena and the way they change from one to another provide some interesting glimpses into cerebral functions.

6. Prevention of Head Injury

Those who are concerned with traffic accidents are constantly reminded of the inadequacy of existing measures to prevent severe injury in many forms of vehicle. As far as the head is concerned, the most obvious need for protection is for motor cyclists (Cairns, 1941). Crash helmets provide a skidding surface by means of which the head can slip along a hard road surface. This, combined with some resilient padding, greatly reduces the deceleration or acceleration forces concerned. The same problem occurs in planning for conditions under which an aircraft can crash on landing without the passengers being killed. Here then is a considerable field for research and application of existing knowledge, which has so far been little developed.

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Book Reviews

Regeneration and Wound-Healing

A. E. Needham. London: Methuen & Co. Ltd., 1952. (Methuen's Monographs on Biological Subjects.) viii + 152 pages; 5 figures. 17 × 11 cm. 8s. 6d.

As the author of this monograph states in his preface, "The marvel of regeneration has aroused the curiosity of naturalists since Aristotle". In recent years interest in this problem has widened, for, as the title of this book indicates, the processes of regeneration in lower animals, as in the case of limbs of Crustacea or Amphibia, have much in common with the problems of compensatory hypertrophy, hyperplasia and wound-healing that are of such practical concern to the human pathologist and the clinician. Mr Needham's approach to his subject has rightly and indeed inevitably been on a broad comparative basis, for the problem is a wide one and, when viewed from the narrower medical standpoint, it must be remembered that our understanding of the nature of regeneration, like that of many of the other fundamental problems in human pathology, will advance most quickly only if we utilize those valuable pieces of information that can so often be obtained from a study of the peculiar ways of life occurring in species far removed from our own.

After a short introduction in which the problem is defined, the author briefly describes the sequence of events in epimorphic regeneration, from the initial wound closure to the differentiation of the newly formed tissue and the final recovery of function. He then deals with the biochemical approach which in recent years has opened up new and promising aspects of the problem. Since the process of regeneration involves the removal of cells damaged by wounding, followed by the production of new cells, proteolysis and protein synthesis are clearly of prime importance, and much work on protein metabolism in this connexion has taken place. The accelerated protein destruction that follows trauma of various kinds, and the "toxic destruction of protein" that occurs during the febrile stage of many acute infections, are conditions well known to the clinician. Although no completely satisfactory explanation has yet been found to account for these aberrations of protein metabolism, the current views on their production and significance are discussed. Further chapters deal with the rate of growth of regenerating tissue, with the causal analysis of regeneration, and with morphallaxis. The monograph closes with an interesting chapter on the distribution and significance of powers of regeneration.

The book is well documented and has a bibliography of over 500 references. Inevitably in a book of this size many aspects of the problem are merely touched on, and one is sometimes left wishing that points that have been mentioned could have been dealt with more fully, if only to explain their relevance to the process under discussion. This, however, is a minor criticism, and the author is to be congratulated on having assembled and presented so clearly so many facts in so few pages. It is a useful, interesting and stimulating book, and can be warmly recommended for a wide range of workers in this difficult field, and indeed for all who are interested in problems of growth and repair.

R. H. S. Thompson

Sympathetic Control of Human Blood Vessels

H. Barcroft & H. J. C. Swan. London: Edward Arnold & Co., 1953. (Monographs of the Physiological Society No. 1.) vii + 165 pages; illustrations. 22 × 14 cm. 18s.

This is the first of a new series of Monographs of the Physiological Society, and sets a high standard.

The authors did not set out to write a comprehensive review, but rather, and probably more usefully, "to present the concept of the control of the blood vessels from the viewpoint of the investigator in this special field". Nevertheless, no special knowledge is demanded of the reader. The problems are simply stated, the evidence is clearly presented, the figures are excellent, and the conclusions are compelling. Professor Henry Barcroft has the ability to ask simple but important questions, and to design experiments which supply the answers. He and his collaborators have been responsible for the greater part of the original work described.

The field covered by the book is in some respects narrower and in others wider than the title indicates. It deals principally and in detail with the circulation through the limbs, while other parts of the peripheral circulation are dealt with only incidentally. The effects of adrenaline and noradrenaline, as well as of the sympathetic nerves and of acute and chronic sympathectomy, are described, and the responses of the vessels of skeletal muscle and skin have been separately analysed on the basis of ingenious experiments.

An interesting chapter is devoted to the action of the sympathetic nerves on the circulation in skeletal muscle during exercise. Evidence is presented which shows that the release of sympathetic vasoconstrictor tone by immersing other limbs in water at 45° C. increases the blood flow through resting muscle; but just as much extra blood flows through the muscles after standardized exercise when the sympathetic tone is released as when it is not. It is suggested that there may be two sets of vessels in muscle—one under the influence of the sympathetic nerves and not affected by metabolic requirements, the other subserving metabolic requirements and not influenced by the sympathetic nerves. There is other evidence to support this view but the authors are wisely cautious in presenting the hypothesis. Our knowledge of the summation of vasodilator responses is rudimentary, and the situation is a complicated one.

Other chapters deal with adrenergic blockade, phaeochrome tumours and the vasovagal syndrome. There is a useful appendix on the history and application of the method of venous occlusion plethysmography, the development and wide use of which owe a great deal to Barcroft and his co-workers.

The book will appeal to a wide range of clinical and scientific readers. The authors, publishers and Physiological Society are to be congratulated on producing a book so remarkably up to date at the time of publication.

A. D. M. Greenfield

Reaction to Injury: Pathology for Students of Disease. Volume II. The Reactions of Submission and Adaptation and the Disease Entities Arising out of their Elaboration

Wiley D. Forbus. London: Baillière, Tindall & Cox Ltd., 1952. xxii + 1110 pages; 836 illustrations. 26 × 18 cm. £7 10s.

Ten years ago, in 1943, the first volume of Dr Wiley D. Forbus's book *Reaction to injury* appeared as a massive work intended for students of disease processes; now he has published an equally large volume which completes the programme set out in the first volume.

Dr Forbus informs us in the preface to the book before us that his work includes all of the material considered essential for a textbook of general pathology, also ". . . the basic clinical material essential to an understanding of clinical disease and its correlation with the corresponding basic pathological processes." He admits that his book is expensive but he modestly points out that "It costs the student the price of three steak dinners, the price of enough gasoline to run his automobile three weeks, the price of a theater and dinner date with his best girl, or the price of one unlucky session with his friends at the green felt-covered table!" Verily, evidence—if more were needed—of the great discrepancy in the standard of living between the American medical student and his needy British cousin.

But putting to one side such frivolities and ignoring for the time being practical objections such as bulk, let us consider what has been the object of Dr Forbus in adding these monumental volumes to the expanding list of textbooks of pathology. Has the author succeeded in his aim and was that aim worth while?

We are left in no doubt about the theme of the enterprise. Dr Forbus maintains that there are three reaction types which constitute

the basis of all forms of disease. Cells and the body as a whole are capable of responding to injury in three ways:

i. by passive submission;
ii. by offering active resistance;
iii. by effecting an adaptation to the changed conditions and circumstances coming from injury.

Anyone who attempts to bring order into a vast conglomeration of observations by the statement of simple guiding principles or laws deserves high praise and will be sure of the gratitude of his colleagues. Dr Forbus is certainly entitled to a big pat on the back for his gallant attempt to live up to his programme and, indeed, he has achieved no little success in a respectable part of his undertaking. No one will question the evidence he brings forward in support of active resistance and adaptation by cells to changed circumstances. Most of us have been brought up with some such idea and have thrilled, at one time or another, to these brilliant generalizations. Dr Forbus is preaching to the converted but it is the attractive, freshening preaching that comes from an enthusiastic teacher. If more evidence be needed in support of that assumption it comes from a scrutiny of the illustrations which, with few exceptions, are altogether admirable and quite often fascinating.

But not everyone will agree with Dr Forbus's concept of passive submission as a fundamental response to injury. To be sure, the expression is used in an almost indeterminate—dare one say vague—fashion in which words may not mean what they seem to say. The reviewer feels that Dr Forbus himself may not be too happy about this grouping, for he is careful to qualify its scope on a number of occasions by admitting that "submission" is very frequently accompanied or masked by other reactions. Many pathologists have been trained in the old-fashioned belief of passive response of cells which emanated from the school of Rudolf Virchow, but familiarity with progress in the biochemistry and physical behaviour of cells encourages revolt from the classical outlook and leads us to believe that many, if not all, of these phenomena are morphological reflections of distorted functional disturbances in the cell. It is here that pathology must set its house in order; to ignore the contributions from other sciences is to court disaster. May we hope that Dr Forbus will attempt some such synthesis as he takes up the labours of a new edition of his book?

The merits, then, of *Reaction to injury* lie in the development of a clear-enough theme which has wide applications to scientific medicine, the attractive descriptions of diseases and disease processes and the skill with which the illustrations are assembled. Amongst the highlights of clever presentation are the chapters on vitamins and hormones, the stimulating section on adaptation pathology, the useful summaries of data on growth and development and the lucid account of the anaemias. The reviewer found the discussion on poisons somewhat disappointing; indeed, the section on poison gases is sadly out of date, for Dr Forbus has completely passed over the outstanding contributions to this topic from the Second World War. May one also draw his attention to the significant contributions of British workers to our understanding of the mode of action of tetanus and botulinum toxins? By the way, it was good to see a portrait of Sir Thomas Barlow included in the account of scurvy rickets.

There are many references assembled at the end of each chapter, some of which seem to have little direct bearing on the corresponding text. The reviewer was shocked to find no mention of Virchow's great paper on embolism nor of Dorothy Russell's monograph on hydrocephalus. British pathology receives scant notice, the references being largely American, with German papers running well behind; this does not seem to fit in very well with Dr Forbus's broad scheme of attack.

G. R. Cameron

Recent Advances in Pathology, by Various Authors

Edited by *Geoffrey Hadfield*. 6th ed. London: J. & A. Churchill Ltd., 1953. x + 374 pages; 86 illustrations. 21 × 14 cm. £1 10s.

In common with other sciences, pathology does not advance equally swiftly in all its divisions, so that first one field, and then another, is the source of new discoveries. This well-known series keeps its readers abreast of recent research in the subject by presenting a group of articles, each written by an authority, on selected aspects of current pathology.

The present volume contains chapters devoted to inflammation and allergy; to the reticulo-endothelial system and some of its diseases; to carcinogenesis; and to selected topics in the cardiovascular, respiratory, hepatic, renal and endocrine systems. The book supplements, rather than supplants, the previous edition.

The individual articles are clearly not designed to be definitive reviews for study by research workers in that particular sphere, but rather as up-to-date accounts of the subject such as might be delivered in the form of a series of lectures to a postgraduate audience. Thus, in general the book stresses what is known rather than unknown, knitting the results of past and present research together to form an intellectually satisfying account that represents the truth as at present understood.

As might be expected, the process is more successful on some topics than on others. The account of hepatitis, for example, is outstandingly good, whereas, in one or two fields where opinion and often observations differ, the author's task is a thankless one.

It is a book that is designed for, and reaches, a wide audience. Not the least important amongst its readers will be those who will find, in the stories of scientific discovery in its pages, a stimulus to embark themselves on a career of research.

W. G. Spector

Disorders of the Blood. Diagnosis: Pathology: Treatment: Technique

Lionel E. H. Whitby & C. J. C. Britton. 7th ed. London: J. & A. Churchill Ltd., 1953. xii + 856 pages; 20 plates; 106 figures. 25 × 16 cm. £3 3s.

When this book first appeared in 1935 it helped more than any other effort to establish haematology as a specialty and at the same time to spread knowledge of the disorders of the blood among the medical profession generally. To give only one example: that the terms "absolute values", mean corpuscular diameter, mean corpuscular volume, etc., with their usefulness in diagnosis and therapy, are now familiar even to the older generation of medical men is undoubtedly due to the impact of this book. There have been new editions every few years and this, the 7th, still conveys the charm and vigour of the first. It is essentially a practical book, designed not only as a textbook to help the student and to prepare the postgraduate for higher qualification but also as a bench book in the laboratory and as a guide at the bedside in diagnosis and in treatment. Faithful to the principle laid down in the preface of its 1st edition it emphasizes how rare is primary disease of the haemopoietic system and how often are changes in the peripheral blood a symptom of a disorder in some other system of the body. Whereas bacteriology or chemical pathology in their entirety may be left to experts, few medical men will be able to afford not to be haematologists of some sort.

The book has been growing with the expanding field of haematology: the previous edition was increased by 85 pages beyond the one before, and now again another 87 pages have been added in this edition. The plates have been increased from 12 to 20 and the figures from 94 to 106. There is a new chapter on the biochemistry and cytochemistry of haemopoiesis, much has been added and altered elsewhere and, in particular, the chapter on technique has been expanded, notably where it deals with coagulation. It becomes doubtful whether it will be possible to expand the book much further without endangering its original character and its usefulness. Some of the growing chapters are already showing a strain, which must be considered a warning signal. To give examples from fields in which the reviewer is interested himself: in the discussion of the possibility of blood groups influencing the incidence of disease, no mention is made of the possible statistical correlation between blood group A and cancer of the stomach. When, regarding racial characteristics, the frequency of Rh genotypes is touched upon, nothing is said about a striking observation in this field: the remarkably high incidence of cDe in American negroes and in Africans south of the Sahara. While it is of course not possible or even desirable to record every such finding, there is a reference to W. C. Boyd's book on this subject that was published in 1939, and is now out of date, but none to his more recent book on human genetics, *Genetics and the races of man*, published in 1950. It is doubtful whether the non-initiated, when reading the paragraph on the distribution of sickle-cell anaemia, will realize that the incidence of 23.7% in western Nigeria does not refer to the anaemia but to the harmless sickle-cell trait. Whilst target cells are found

in Mediterranean anaemia, when haemoglobin C is present in the red cells, it has been known for some time that the abnormal haemoglobin in the anaemia is not haemoglobin C but foetal haemoglobin. All these are minor blemishes but one could add further examples.

"Whitby and Britton" is a classic, undoubtedly a new edition will again be needed in a few years' time; and one might well ask whether the authors would not render a service to their book and to all of us who cherish it, by developing it into a work comprising two or even three volumes, each of them more like the "Whitby and Britton" of 18 years ago.

H. Lehmann

Immunochemistry

Organized and edited by *R. T. Williams*. Cambridge: University Press, 1953. (Biochemical Society Symposia No. 10.) 94 pages; figures. 25 × 16 cm. 12s. 6d.

In recent years the Biochemical Society has organized Symposia at which specialists have been invited to review particular aspects of biochemistry. These Symposia, some of the earlier of which have been reviewed in this Bulletin (*Brit. med. Bull.* 1952, **8**, 276), have proved popular and successful, for they have provided up-to-date and often stimulating surveys of current research in many important branches of biochemistry. The present review of immunochemistry is a worthy member of this useful series.

One of the objects of the organizers of Symposia of this type is to stimulate free discussion by specialists and non-specialists, but unfortunately this object is not always achieved. Admittedly it is often extremely difficult to persuade contributors to these discussions to provide suitable abstracts of their contributions, but it might add appreciably to the value of future monographs in this series if brief mention were made of the more important parts of the discussions.

The present is an opportune time to review the important contributions which chemists have made in the study of some of the complex problems of immunology, and it is fitting, because of his outstanding contributions in this field, that J. R. Marrack should have written the introduction to this monograph and also an article on immunity reactions in relation to the structure of proteins. In the latter he discusses, on the reasonable assumption that molecules of antibodies and protein antigens have a coiled peptide chain structure, present-day views on the nature of the specific combining groups of antibodies and native protein antigens, and on the formation of antigen-antibody precipitates.

The advantages gained by using certain enzymes as antigens are mentioned by B. Cinader in his review of antigen-antibody interaction using enzymes as antigens, and W. E. van Heyningen gives a most useful account of the immunology of toxic proteins in his review of the reactions of toxins with antitoxins. That immunologists are not neglecting modern techniques is well evident from the excellent account by G. E. Francis of the use of isotopically-labelled antigens and antibodies in the study of the antigen-antibody reactions and the fate of injected protein antigens.

P. G. H. Gell discusses a few aspects of hypersensitivity, including the view, largely advanced and developed by Landsteiner and his colleagues, that many chemical sensitizers act after conjugation with body proteins. Another interesting chapter is written by M. Stacey, who reviews the role of carbohydrates in immunochemistry.

In his article on the relation of complement to the nitrogen bound by antigen-antibody precipitates, R. G. S. Johns has attempted to simplify matters by postulating that complement is a single molecule. Such a conception must necessarily ignore extensive evidence that the complement system contains four components which can in some cases be separately inactivated and some of which can be separated in a highly purified form. Most biochemists would, indeed, find it hard to accept the conception of a single complement *molecule* which can be divided, by treatment with neutral salts or very dilute ammonia or even by simple dialysis against water, into two or more parts which would at once reunite, with regeneration of complement activity, when mixed in 0.9% NaCl at pH 7.5. The author refers to the speculative nature of this hypothesis, and if this very drastic attempt at simplification is not taken too seriously, it may function as a useful stimulus, provoking others to renew and intensify their efforts to solve one of the most fascinating problems of immunochemistry.

A. Wormall

The Faber Medical Dictionary

Edited by *Cecil Wakeley*. London: Faber & Faber Ltd., 1953. vii + 471 pages; 6 illustrations. 28 × 19 cm. £2 5s.

What are the criteria by which a medical dictionary should be judged? According to the reviewer's ideas they are: accuracy of definition—and definition is one of the most difficult of accomplishments—and succinctness or brevity without sacrifice of lucidity. The pitfalls besetting the compiler are omission of important matters, erroneous information and misprints.

Without any beating about the bush we may say that the present work is one of the best medical dictionaries in the reviewer's knowledge, and the editor, Sir Cecil Wakeley, and his able panel of coadjutors are to be heartily congratulated on the accomplishment of a very difficult task. For each entry, apart from the definition, the author gives the correct pronunciation and the derivation of the names; and much interest lies in the inclusion of the dates of birth and death of physicians, surgeons, gynaecologists and others whose names are connected with symptoms, discoveries, operations, apparatus, etc. Moreover, not merely definitions of words and terms are given, but also certain details, where this is possible in a few words; and useful cross-references are not omitted, e.g. under "chloromycetin", the reader is referred to "chloramphenicol", and, for adrenaline, the formula, the full chemical name and the synthetic preparation are given.

In short, the work is so good that we feel that neither the editor nor his staff will take umbrage if we take up a few points in accordance with the criteria as stated above. Most of the corrections and suggestions could easily be printed on a single page of addenda or corrigenda, and, as fresh terms arise, addenda will be called for to bring the work up to date from time to time, and a loose-leaf insertion will obviate expensive reprinting. That it is at present well up to date will be obvious to anyone who consults the dictionary; there is no need to cite examples. What better or more succinct definition could be found for dipsomania than "periodic alcoholism"? The entry for "anaemia" is really an instructive treatise *in parvo*, as also is that for "aphasia". To define the D of ADT in a placebo prescription as "What you please" is a very happy euphemism, and the accounts of arteries, veins, nerves, muscles and ligaments with their diagrams constitute almost a treatise on, at any rate a students' aid to, anatomy. "Nyctalopia" is another example: it is defined correctly as "night blindness", with an explanation why some writers ascribe the term to "day blindness". We are glad to see that there is no attempt to perpetuate T.B. as a synonym of tuberculosis, as is often seen even in medical papers and used colloquially by medical men as well as by the laity, and that "tuberculous" is stressed as the correct adjective, not "tubercular".

A few words on mistakes and omissions are called for; these are not as many as might be expected in a work of this size. A good deal is written under "alpha" and its uses in physics, chemistry, bacteriology and pathology, but no mention is made of alpha-privativum, though its negative meaning appears in scores of examples. The term "bacterium" is used to cover any schizomycete, and "bacillus" is defined as a rod-shaped bacterium. Nothing is said of sporing organisms to which, in bacteriology, the term "bacillus" is restricted, e.g. *Bacillus anthracis*, *Bacillus tetani*, though some distinctions are made by saying: "B. coli: see bacterium coli . . . B. typhosus: see salmonella typhi", etc.; but leprosy is said to be caused by *Bacillus leprae* instead of *Mycobacterium leprae*, though tuberculosis is rightly ascribed to *Mycobacterium tuberculosis*. Antivenin is defined as "immune serum . . . against snake poison", omitting the fact that antisera are in common use against scorpion-stings and spider-bites and the venoms they inject. *Shigella* is defined as "dysentery-causing bacilli" and under "dysentery" the causes are given as "*Bacillus dysenteriae Shiga*", "*Bacillus paradysenteriae Flexner*", "Sonne's bacillus". Other mistakes calling for correction include "Kitasato's bacillus": Kitasato was not the discoverer of *Pasteurella pestis*; Kitasato's organism was gram-positive and motile and had nothing to do with the causative organism of plague. *Pasteurella pestis* is gram-negative and non-motile and was discovered by Yersin who, incidentally, was *not* a Russian. Again, "Queensland fever" is not the correct name for Q fever caused by *Rickettsia burneti*. It is true that Q fever was first recorded in Queensland but the initial Q was given to it to signify "Query fever" because of the doubt concerning its nature and causation. It occurs also in Spain, Britain, America, and has in fact a wide distribution.

Pinta is not " a tropical skin disease . . . due to various species of chromogenic fungi ". As long ago as 1927 its spirochaetal (treponemal) aetiology was suggested and this was proved in 1939; the cause is *Treponema herejoni*. Lastly, " Seidelin's bodies ": the author has slipped badly here. Harald Seidelin is not a " contemporary British physician ". He was a Dane, a pathologist, who worked in Mexico, the West Indies and Liverpool. Incidentally, he worked for some time in the reviewer's laboratory in Jamaica. The term " paraplasm " is mentioned in this dictionary, but not the fact that Seidelin named his " bodies " *Paraplasma flavigenum*, in the mistaken belief that they were the cause of yellow fever, a belief disproved by Wenyon and Low who showed them to be present in the corpuscles of normal guinea-pigs. Seidelin died some eight to ten years ago.

Certain omissions call for insertion: *Lymnaea*, the host of *Fasciola hepatica*; *Plasmodium ovale* among the human malaria parasites, discovered by Stephens in 1922. No reference is to be found to the malaria parasites of birds or monkeys, nor to Henry's melano-flocculation test for malaria, nor to Maurer's dots or clefts. Fluorine is mentioned, but not fluorosis or the effect of fluorine on the teeth. Premunition is not referred to at all, nor is pyretotherapy and the use of malaria in the treatment of neurosyphilis—a most important omission. Latah finds no place, nor does toxoplasmosis. Zoophilism is defined as " love of animals ", without reference to the liking of mosquitoes for animal blood in preference to the blood of man (anthropophilism also is omitted), and to the use of this knowledge as an aid to malaria prevention. Anatoxin is mentioned but not anavenin, nor do we find Romaña's sign of American trypanosomiasis—Chagas' disease. The viscerotome, used originally for taking specimens of liver tissue in deaths from suspected yellow fever, but now for many other purposes, is not referred to. Favus is included, but not favism, the disease caused by inhaling the pollen, or, more often, by eating the bean, of *Vicia faba*; *Bartonella bacilliformis* is given as the cause of Oroya fever and Carrión's disease, but not of verruga peruana. Bejel, the non-venereal syphilis of the East, finds no place; B.M.R. (basal metabolic rate) is found, but not B.S.R. (blood sedimentation rate); pyomyositis is another omission; D.D.T. is given but not D.D.D. (dichlorodiphenyl dichlorethane); D.D.S. is given as " Doctor of Dental Surgery ", but not as dichlorodiphenyl sulphone, nor sulphetrone, used so widely and successfully in leprosy; we look in vain for gammexane or benzene hexachloride (B.H.C.), the much-used insecticide. The relatively rare coccidioidal granuloma finds place but not the more common granuloma venereum, though lymphogranuloma inguinale is here. " Antibiotic " in general is mentioned and one or two antibiotics in particular, but there is no word of the distinction between members of the bactericidal group, such as penicillin, streptomycin and neomycin, and members of the bacteriostatic group, such as aureomycin, terramycin and chloramphenicol. So important a matter as xenodiagnosis is passed over; Leishman and Giemsa stains are mentioned but not Romanowsky's, on which both were based.

Misprints are remarkably few: we may mention *Ankylostoma canicum* for *Ancylostoma caninum*; pituitry (p. 199); *Fasciolopsis burki* for *buski* (p. 158), and " zoospermia " is out of place and should come six places further down to keep alphabetical order.

The reviewer would like to end by repeating that these changes could all be embodied in a page insertion, and that they are put forward in no carping spirit, but solely with the view of making an excellent work a little more complete.

H. Harold Scott

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